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RESEARCH ARTICLE

COMPARATIVE STUDY BETWEEN DRINKING WATER AND DISTILLED WATER AS SOLVENTS FOR EXTENDER OF BUCK SEMEN

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ABSTRACT

This study was conducted to determine the effect of drinking water as solvent for extender on motility of buck sperm. Semen was collected by artificial vagina from five bucks. Four different source of drinking water (Zamzam, Hana, Nova and Aqafina water) and distilled water were used as solvents in Tris-egg yolk extender. So five extenders were formed Zamzam, Hana, Nova, Aqafina and distilled extenders. Sperm have the longest life and motility when using Zamzam extender (12 days). While, the use of distilled extender caused reduction in survival rate and motility of sperm (5.5 days). drinking water such as Zamzam water might be use as solvent in Tris-egg yolk extender for chilled semen of buck. So, we have to reconsider the composition of extender such as solvents and elements (calcium and potassium).

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INTRODUCTION

Since biological research that established, we use distilled water as a solvent for salts and sugars (Kozisek, 2006). Where we were studying the presence of these composition on the vitality of living cells or to be used for culturing cells or bacteria present in nature. One of living cells that have been studied is sperm. Which it produced a lot of extenders for elongation vitality of sperm for hours, days, or years (freezing extenders). The basic substrate to form the extender is distilled water. Knowing that distilled water has very small proportions of minerals and compounds (Kozisek, 2006). As for drinking water , they contain a high concentration of salts and heavy and trace elements (Sivasankar *et al*., 2014). Therefore, the distilled water was not suited to the use to drink for lack of some elements that are important for a healthy body (Kozisek, 2006). It is more commonly used water in Saudi Arabia Zamzam, Hana, Nova and Aqafina water. It is known that sperm motility is a central component of male fertility because of its importance on migration in the genital tract and gamete interaction for fertilization (Suarez and Pacey, 2006). This study is based on the study of the effect of the use of drinking water such as Zamzam, Hana, Nova and Aqafina water instead of distilled water in extenders on the sperm motiltiy of buck semen.

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MATERIALS AND METHODS

Animals

This study was carried out in the Center of Agricultural and Veterinary Research at Qassim University. Five bucks (Damascus X Ardi goats) at least 1.5 year were selected. They are healthy and feed on concentrated and dry green clover twice a day. These males were training for semen collection.

Semen collection

The semen was collected twice a week by using artificial vagina. After semen collection, the semen was directly assessed. The semen that is used in the study needed to pass the terms of the evaluation, which include the following: volume (0.5 -1 ml), color (creamy or milky), wave motion (4-5 score), the individual movement (at least 70%), the proportion of live/dead sperm using dye eosin and nigrosin stain (not less than 70%), sperm concentration using haemocytometer counting (at least 900 X 10^6 /ml), and finally the presence of abnormalities in sperm shape (not more than 10%). after the acceptance of the evaluation, one part of semen was diluted

with a 3 part of diluents. Motility of sperm was recorded every 12 hours until reach individual movement to zero%.

Diluents

Specifically, Tris-based extenders containing low concentrations of egg yolk are most commonly used for cryopreserving and chilled semen from buck (Purdy, 2006). The diluent composed 3.63 g of Tris, 1.99 g of citric acid, 0.50 g of fructose, 2.5 ml of egg yolk, 100 000 IU of penicillin and 125 µg of streptomycin. all components were dissolved in 100 ml of distilled water (control diluent). The formation of other diluents which contain the same proportion of the above materials, but dissolved in drinking water from different source (Zamzam, Hana, Nova and aqafina water). The components that disloved in the Zamzam water produced Zamzam diluent, dissloved in Aqfina water produced Aqafina diluent, dissloved in Hana water produced hana diluent, or dissloved in Nova water produced Nova diluent. The chemical composition of drinking water are shown in the following table (1).

Table 1. Chemical Analysis of Hana, Nova, Aqafina and Zamzam water

Elements	Hana*	Nova*	Aqafina*	Zamzam*
Na	22	17	16	135
Ca	8	11	< 5	96
Mg	3	3.4	13	38.88
K	1	1.2	1	43.2
HCO_3	26	26	1.3	192.4
Cl	40	19	27.5	159.7
Fl	1	3	1	0.68
NO_3	3	3	< 0.1	126.1
SO_4	32	26	51	123.3
pH	7.2	7.4	7.0	7.90
TDS	127	120	110	840

Note: all values except pH are in mg/l.

*Source from Hana, Nova and Aqafina company. While chemical composition of Zamzam water from Al-Zuhair and Khounganian (2006)

Sperm progressive motility

The percentage of sperm progressive motility was assessed by light microscopy at 400 magnification with a heating stage at 37 ºC. Sperm was considered motile and counted if they had a significant motive ability in the linear orientation under microscopic view. Ten microliters of sperm sample were deposited and covered with a cover slip on a pre-warmed hemocytometer.

Statistical Analysis

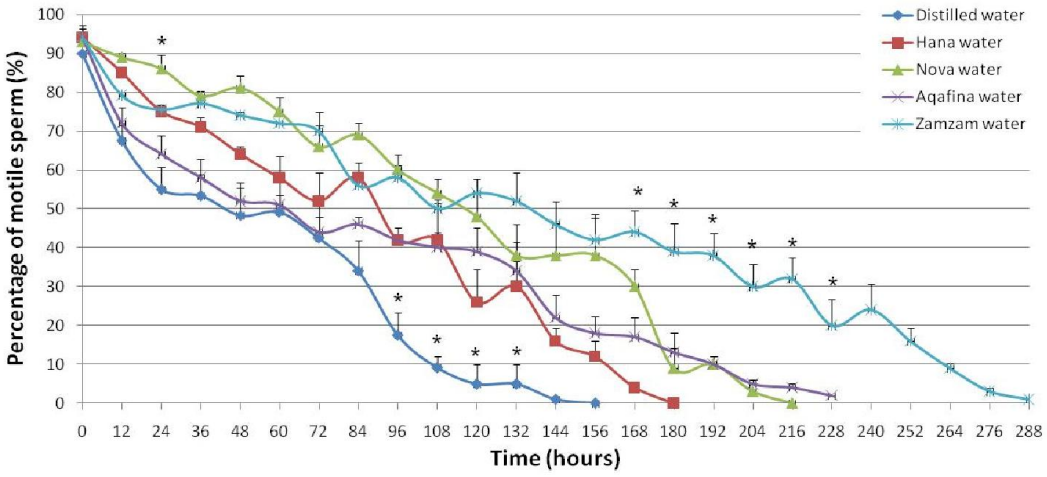
Data were analyzed using a statistical software SPSS release 16.0. Because the data were not normally distributed, a kruskal-Wallis one-way ANOVA (non parametric statistical test) was used to test for the presence of significant difference among all five groups. Data are presented as arithmetic mean ± SEM.

RESULTS

Figure (1) shows the proportion of sperm movement with time. Whenever the time period increased, the percentage of sperm motility was decreased. The percentage of sperm motility was more than 90 % at 0 hour (time add diluents). However, the percentage of sperm motility dropped to less than 50 % at 48, 96, 120, 72 and 144 hours in distilled, Hana, Nova, Aqafina and Zamzam diluents, respectively. In Zamzam diluents, the percentage of sperm motility at hour 168, 180, 192, 204, 216 and 228 were 44±5.5, 39±7.3, 38±5.8, 30±5.8, 32±5.4 and 20±6.7 %, respectively. The difference was significantly higher (P< 0.05) in Zamzam diluents compared to other groups. While, the percentage of sperm motility at hour 96, 108, 120 and 132 in distilled diluents were 17.5±5.7, 9±3, 5±5 and 5±5, respectively.

Table 2. survival rate of sperm in days through percentage of sperm motility

Percentage of motile sperm (%)	Time from adding extender to cessation motility of sperm (days)				
	Distilled water	Hana water	Nova water	Aqafina water	Zamzam water
80≤	-	0.5	1	-	-
70- 79	-	1.5	2.5	0.5	3
60-69	0.5	2	4	1	
50-59	1	3.5	4.5	3	5.5
40-49	3	4.5	5	4.5	7
30-39	3.5	5.5	7	5.5	9
20-29	-	-	-	6	10
10-19	4	6.5	8	8	10.5
1-9	5.5	7.5	8.5	9.5	12



*Significant difference (p<0.05) among groups.

Figure 1. Effect of adding distilled and drinking water in the Tris diluents on the vitality of buck

However, the difference was significantly lower (P< 0.05) compared to other groups. It is noted that the sperm which diluted in Zamzam diluents were more vital and prolong her life for reaching 288 hours. While the use extender contains distilled water, sperm motility was reduced quickly by the time and reached 156 hours. Other diluents using Hana, Nova and Aqafina diluents prolong life of sperm for 180 , 216 and 228 hours, respectively.

In Table (2) shows the duration of sperm survival rate in days through percentage of sperm motility. Sperm with 50-59 % of the motility was at Day 5.5, 3, 4.5 and 3.5 and 1 when diluted in Zamzam, Aqafina, Nova, Hana and distilled diluents, respectively. However, 30-39 % of the sperm motility was at Day 3.5 and 5.5, 7, 5.5 and 9 when using distilled water, Hana, Nova, Aqafina and Zamzam diluents. So by the results, it is noted that the use of Zamzam water as solvent for extender was the best in sustaining the vitality of sperm and prolong her life period compared to use distilled water or any other kinds of drinking water.

DISCUSSION

This study describes the use of drinking water as solvents in extenders that are used in semen preservation. The chemical composition of water close to the chemical composition of semen. This is normal because of the animal that deals with groundwater and by following reflect the chemical composition of semen. Through the results, Zamzam water gave the best extend the life of sperm. Zamzam water is used for more than 4000 years and to some extent now (Al-Zuhair and Khounganian, 2006). The one thing that is different in Zamzam water compared to other water it contains double the amount of salt (Table 1). These elements such as calcium, chlorine, sodium, potassium and phosphorus and compounds such as sulfate, bicarbonate, nitrate. These elements are very important for vitality, metabolism and movement of sperm. Especially in the presence of calcium. Calcium component of the most influential of the vitality of sperm elements considered. This element is present in abundance in the water of Zamzam. Calcium is acknowledged to be a major regulator of sperm physiology, including epididymal maturation (Magnus *et al*., 1990), motility (Marquez and Suarez, 2007), membrane function (Magnus *et al*., 1990), metabolism (Magnus *et al*., 1990) and induces increased respiration and motility (Tateno *et al*., 2013) in mammalian spermatozoa. It is involved in the processes of capacitation, acrosome reaction and fertilization (Linares-Hernández, *et al*., 1998) including the induction of hyper activated motility which appears to be a prerequisite for egg penetration (Linares-Hernández, *et al*., 1998). Elevations of flagellar Ca2 seem to be responsible for a hyper activated form of sperm motility (Marquez and Suarez, 2007) first seen in the female. Because millimolar concentrations of extracellular calcium are needed to develop and maintain hyper activated motility in vitro, plasma membrane channels have been proposed to mediate the apparent Ca2 mobilization (Timothy *et al*., 2003). Since the calcium component increases the metabolism inside the sperm means that it works to keep the vitality and movement of sperm for longer. This explains through the use of distilled water, where it is almost empty of elements and compounds, and by following sperm get items from the seminal plasma and through the addition of salts or compounds diluted to extenders such Tris, citric acid and fructose. That's why, using distilled water, vitality of sperm lose rapidly over 6-7 days. Progesterone (P) and zona pellucida are known to induce acrosome reaction in human sperm by increasing cytosolic calcium. High concentrations of potassium ions (K+) improve the rate of acrosome reaction in human sperm in vitro. It would appear that human sperm plasma membrane possess different Ca2+ channels responsive to P and K+ (Kumar *et al*., 2000).

Conclusion

So through these results we must reconsider the structure of extenders used to preserve the semen. For example add two elements like calcium and potassium in the form of compounds.

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