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Original article

Comparison of sperm characteristics, antioxidant and oxidant levels of frozen semen produced in bulls'0.5 and 0.25 ml straws

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Abstract

Background: The aim of this study was to compare the quality of bull sperm produced in 0.5 and 0.25 ml semen straws before and after freezing.

Methods: In this experimental study, semen samples were collected from 12 dual purpose Simmental (Fleckvieh) bull kept at Iran Simmental Cattle Breeding Center during a two-month period. Each ejaculate was divided into two equal portions. Then, the formulation and freezing processes were performed separately for each type of straw. Sperm qualitative characteristics, antioxidant and oxidant levels of frozen semen produced in bulls' 0.5 and 0.25 were compared.

Results: The results showed that all sperm quality parameters in 0.25 ml straws were better than 0.5 ml before and after freezing. Catalase (CAT) activity was higher in 0.5 ml straws than 0.25 one, but it was not statistically significant. Malondialdehyde (MDA) levels were lower in 0.5 ml straws than 0.25 ml straws, but the differences were not statically significant. Moreover, no remarkable difference was observed in the activity of superoxide dismutase (SOD) activity enzyme.

Conclusion: Based on the findings of the present study and with the aim of reducing the production costs, it is recommended that bull sperm production centers and herdsman inseminators use 0.25 ml straws for semen freezing and cattle artificial insemination.

Keywords: Antioxidants, Cattle, Oxidants, Semen, Semen Preservation, Spermatozoa

Introduction

Cattle artificial insemination (AI) in bovine with frozen-thawed semen packed in both 0.5ml and 0.25ml straws is a universally accepted method. However, all processes of production, thawing and the insemination of the frozen semen persuade adverse effects on the ultra-structure, biochemistry and all semen quality parameters, which can lead to a decrease in motility, membrane integrity and fertilizing ability (1). Numerous investigations are ongoing to improve frozen semen quality, the storage of produced semen, the handling of packed semen while AI, the conception rate as well as a decrease in production cost (2-8). Trying to achieve the highest quality with the least damage on produced frozen semen, we need to consider the following topics: the cryoprotectant materials and their component rations, the types of extenders, the dilution rate, the packaging methods, and the freezing rate.

The packaging of frozen samples for storage must meet several criteria, such as a large surface to enable a uniform cooling rate of the sample, the proper heat exchange properties, the ease in labeling and secure sealing, and the availability in small units. The methods such as semen glass ampoules, pellets of dry ice and others were plenty valuable for packaging and freezing sperm and had a remarkable role in the advancement of cryobiology science. Since Eduard Sorensen invented plastic straws and Robert Cassou modified them and introduced the 0.5 ml and 0.25 ml

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polyvinylchloride straws, they have almost become a part of the history of assisted reproductive technology (9). Cassou's 0.5 ml straws with a diameter of 2.8 mm and the volume of 0.5 ml were replaced with larger Sorenson's straws with a diameter of 4.2 mm and volume of 1.2 ml. Furthermore, Cassou introduced the mini straws with the volume 0.25 ml. Both sizes are still in use today (10).

Today, it is globally accepted that the 0.25 ml straw is the predominate straw for the production of bovine frozen semen owing to some economic advantages including: the saving of liquid nitrogen, container storage, extender and antibiotic consumption, reducing production, storage and shipping costs (11-13). As 0.25 ml straws have a higher surface to volume ratio rather than the 0.5 ml one, the temperature exchange between spermatozoa and nitrogen vapor is faster and provides homogeneous freezing and thawing temperature exchange throughout the straw (14). This can act as a double-edged sword that may have both favorable and unfavorable consequences. Although this may be an advantage in some freezing protocols with a slow freezing rate, it could not be deemed as superiority when it comes to accelerated freezing rate in order to provide optimum freezing in 0.5 ml straws (12). Regarding 0.5 ml straws, the advantages of handling and reading printed straws could be easy and may be more resistant to physical damage during storage in liquid nitrogen container (11) . On the other hand, there are some research studies recommending that 0.25 ml straws have positive impacts on post-thaw sperm viability when the cooling process is optimized and thawing rate is accelerated. For this reason, since 2002, the accepted straw type for packaging and freezing of ram semen in Norway has been the 0.25 ml straw (15).

Reactive oxygen species (ROS) at the physiological levels may have effects on acrosome reaction, hyperactivation and sperm-oocyte binding. However, excessive ROS can destroy sperm functions and sperm DNA integrity during cryopreservation, as more ROS generation could cause oxidative stress (16, 17). It is worth mentioning that scanty studies have been carried out so far to determine the antioxidants and lipid peroxidation levels of semen in two standard types of straw (0.25 and 0.5 ml) after frozen thawed in bulls.

Hence, the aim of the present study is to compare semen quality, antioxidants, and lipid peroxidation levels after thaw packed and frozen in two standard types of straw (0.25 and 0.5 ml) in dual purpose German Simmental (Fleckvieh) bulls. Our hypothesis is that the use of 0.25 ml straws for bull semen freezing is a better choice compared with 0.5 ml straws in terms of both the quality and cost effectiveness for both the producers and consumers.

Materials & Methods

Animals

This study was performed on 13 healthy breeding bulls (age range, 3-7 years), Fleckvieh from July to September (2019). The protocol was performed in accordance with the ARRIVE (Animal Research: Reporting of In vivo Experiments) guidelines. The Animal Research Committee of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Mazandaran approved the study. Semen samples were collected according to routine weekly production at Iran Simmental Cattle Breeding Center (height above sea level: 47m, 36° 28′ 11″ N, 52° 21′ 3″ E) with the total number of 78 samples (three ejaculations for each bull). The bulls were fed three times per day based on standard Fleckvieh bulls' daily diet formula, which is mentioned in Table 1.

Semen collection

Semen ejaculates were collected by artificial vagina (AV) in accordance with routine sample collection in AI centers. Sexual preparation of bulls was done by 10 min of standing in collection area after three false mounts. AV was pre-warmed in oven (Memmert, Buechenbach, Germany) at 46 ˚C before usage. Immediately after collection, semen volume and concentration were measured by a sterile graduated glass vial and Photometer SDM (Minitube, Tiefenbach, Germany) calibrated for bull sperm cell counting, respectively. Each ejaculate was divided into two equal parts for separate formulation, packing and freezing.

Semen processing and freezing

In order to estimate the fresh sperm motility, two drops of diluted semen were put on glass slide and analyzed using a binocular Microscope MBL 2000 phase contrast microscope (Minitube, Tiefenbach, Germany) equipped with warm stage at magnification of ×200. Only the progressive motile spermatozoa were considered. It should be noted that the basis of the sperm progressive motility (PM), the percentage estimation for formulation, was identical for both 0.5 ml and 0.25 ml straws and was detected by the eye, as

this method allowed the number of spermatozoa to become equal in both straws. Further investigations such as computer assisted sperm analysis (CASA), motility parameters and other semen characteristics were done after the formulation.

One step dilation method (room temperature semen packaging) was performed for sperm freezing, which is briefly explained as follows: 1) The preparation of pre-extender dilution: gently adding extender (Steridyl CSS, Minitube, Tiefenbach, Germany) to the semen (with ratio of 1:1) and placing it in a water bath at 34 ˚C for 10 min. 2) The calculation of final extender volume was done via following formula; the number of doses = (semen volume * semen concentration * progressive motile sperm * morphologically normal sperm) / (sperm per dose [15 million]). 3) The preparation of the final solution with the addition of pre-extender to final calculated extender volume and then leaving it at room temperature (20-24˚C) for 15 min. Since the volume of 0.25 ml straws are half the size of 0.5 ones, the amount of extender required for 0.25 ml straws are half of the 0.5 ml straws with this formulation. 4) The separate packaging of the 0.25 and 0.5 ml straws (Minitube, Slovakia) with MPP Uno automated filling and sealing machine (Minitube, Tiefenbach, Germany). 5) Placing the packed straws at 4˚C for 3 hours in the refrigerator in order to reach the sperm equilibrium stage. 6) The separate freezing of the straws with putting 0.25 ml straws at -120˚C for 8 min and the 0.5 ones for 10min in MT freezer freezing device (Minitube, Tiefenbach, Germany) in accordance with manufacture instructions 7) Storing the frozen semen in liquid nitrogen containers.

Sperm viability and morphology evaluation

Eosin-nigrosin (Minitube, Tiefenbach, Germany) staining was used for viability and morphological abnormality assessment by examining 200 sperm per sample under 400 x magnification with a phase contrast microscope (MBL 2000; Minitube, Tiefenbach, Germany).

Assessment of membrane integrity

In order to predict sperm membrane integrity, hypo-osmotic swelling test (HOST) was carried out. HOST can evaluate the ability of the sperm membrane to maintain equilibrium between the spermatozoa and its environment. Due to hypo-osmotic solution, the incursion of the fluid causes the sperm tail to coil or swell. The higher the percentage of sperm with coiled tail, the healthier and more functional sperm membrane. For each sample, at least 200 spermatozoa were analyzed with 400x magnification under a phase contrast microscope.

Computer assisted sperm analysis (CASA)

Sperm motility parameters were analyzed before and after freezing by CASA system (Hooshmand Fanavar, Tehran, Iran). The parameters included PM, curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), the mean angular displacement (MAD), lateral head displacement (ALH), beat cross frequency (BCF), linearity (LIN [VSL/VCL]), wobble (WOB [VAP/VCL]), and straightness (STR [VSL / VAP]). All analyses were carried out by light microscope equipped with hot plate, which maintained the sample at 37˚C in a dedicated chamber (Sperm meter, Depth 10 micron, Surface Graticule, 100x 0.1 square/mm diameter) to avoid the reduction of sperm quality during assessment.

Measurements of CAT, SOD and Malondialdehyde

The formation of thiobarbituric acid (TBA) in semen was assessed for concentration evaluation of malondialdehyde (MDA) using an original method. Briefly, semen samples were mixed with 20% trichloroacetic acid and the mixture was centrifuged. Then, the supernatant was heated with thiobarbituric acid at 90 ° C. Its absorbance was measured at 532 nm. The values were expressed as nmoles MDA/mg using a molar extinction coefficient of 1.56 × 105 M-1. Commercially available kits (ZellBio GmbH, Germany) were used to estimate CAT and SOD in the seminal plasma following the manufacturer's guidelines.

Statistical analysis

SPSS V.24 was used for all statistical analyses. Non-normal data were normalized using Johnson translation in Minitab (version 19.0, Minitab Ltd, Coventry, UK) ($P \leq 0.05$). First of all, independent T-test was used to compare 0.5 ml with 0.25 ml straw. After that, the PM and progressive motility of frozen thawed semen (PMFT) was indicated as the main marker for pre and post thawing bull sperm capacity evaluation, respectively (18, 19). In pre frozen-thawed sperm parameter examination, all samples were divided into three main groups: < 75 %, 75 – 85 % and > 85 %. Also, frozen thawed semen samples were classified into< 40%, 40 – 50 % and > 50% groups.

Multivariate analysis of variance (MANOVA) was conducted for Straw-Type * PM-ratio, Straw-Type * PMFT-ratio and Straw-Type * PMFT-ratio * PM-ratio.

Table 1. The composition and amount of daily meals for the Fleckvieh bulls

Ingredient	Amount	Chemical composition					
	kg	Crude protein (%)	NDF[3] (%)	ADF[4] (%)	Fat (%)	Ash (%)	Dry matter (%)
Concentrate mix [1]	9	14.68	16.8	13.1	3.2	7.4	89.2
Silage	18	8.5	54.5	32.7	1.8	5.7	25
Hay	3	16.8	44.7	34.6	2.5	9.7	1.88
Straw	*Ad libitum*	3.9	70.3	45.5	1.1	9.8	94.8

[1] Ca 0.74%, P 0.53%, Na 0.49%, Mg 0.29%, Zn 375 ppm, Mn 381.44 ppm, Co 1.01 ppm, Se 2.75 and vitamin additives (vitamin A 7500 U/kg, vitamin D3 1000 U/kg, vitamin E 10 mg/kg).
[2] Mg 2.1%, Na 7%, Fe 355 mg/kg, Zn 1560 Mg/kg, Cu 390 mg/kg, Mn 1560 mg/kg, Se 7.5 mg/kg, Co 3 mg/kg, I 15.5 mg/kg.
[3] NDF: neutral detergent fiber, [4] ADF: acid detergent fiber
Source: Data calculated by animal nutrition laboratory, Faculty of Agriculture, University of Tehran, Iran.

Results

A significant difference was detected in the straw type and PM-ratio when evaluated by using MANOVA, but no statistically significant difference was seen in straw-type * PM-ratio assessment. Interestingly, the same document was detected in straw-type, PMFT-ratio and straw-type * PMFT-ratio assessment in frozen thawed experiment. Overall, no statistically significant effect was detected in straw-type * PMFT-ratio * PM-ratio groups, which might indicate PM as well as straw-type rare effect on semen parameters. All in all, no statistically significant influence was observed in the combination of all factors (Table 2).

Table 2. Antioxidants and oxidants of semen at and post-thaw Frozen thawed semen packed in 0.5 and 0.25 ml straws

Group	Before freezing			Frozen thawed semen		
	Straw-type	PM-ratio	Straw-type * PM-ratio	Straw-type	PM-ratio	Straw-type * PM-ratio
Wilks' Lambda	0.22	0.10	0.19	0.22	0.10	0.19
F	3.93	2.30	1.39	6.52	6.27	1.40
Df	35.00	70.00	70.00	20.00	40.00	40.00
P	0.00	0.00	0.07	0.00	0.00	0.29
Eta2	0.77	0.67	0.55	0.77	0.67	0.55
Observed power*	1.00	1.00	0.99	1.00	1.00	0.92

The comparison of semen quality parameters in 0.25 and 0.5 ml straws showed that there were many statistically significant differences in both fresh and frozen thawed semen groups. Regarding before freezing samples, PM ($P<0.01$), VCL ($P<0.05$), VAP ($P<0.05$), MAD ($P<0.05$), LIN ($P<0.01$), WOB ($P<0.01$) and STR ($P<0.01$) were higher and BCF ($P<0.05$) was lower significantly in 0.25 ml straws compared with 0.5 ml straws. In addition, viability and HOST were significantly higher in 0.25 ml straws ($P<0.05$). Furthermore, the percentage of total abnormal morphology ($P<0.05$) and the abnormality in head ($P<0.05$) and mid piece ($P<0.01$) were significantly lower in 0.25 ml straws. In frozen thawed

semen, PM, LIN, WOB and STR were higher (P< 0.01) and BCF (P< 0.01) was significantly lower in 0.25 ml straws in comparison with 0.5 ml ones. Moreover, the total sperm abnormal morphology and the abnormality in head and mid piece were significantly lower in 0.25 ml straws (P< 0.01) (Table3).

Table 2. Comparison of semen quality parameters in fresh and frozen thawed semen which separately packed in 0.5 and 0.25 ml straw

Variable	Before freezing		Frozen thawed semen	
	0.5 ml Straw Mean ± S.E	0.25 ml straw Mean ± S.E	0.5 ml Straw Mean ± S.E	0.25 ml straw Mean ± S.E
Sperm progressive Motility (%)	66.8± 2.3**	79.4 ± 1.0**	34.5 ± 1.7**	46.29 ± 1.90**
Curvilinear velocity (μm/s)	37.7 ± 2.3*	46. 7 ± 2.2*	15.6 ± 0.9	17.7 ± 1.0
Straight line Velocity(μm/s)	21.3 ± 1.6	25.4 ± 1.4	8.5 ± 0.5	9.0 ± 0.6
Average path velocity (μm/s)	27.0 ± 1.8*	31.9 ± 1.5*	11.0 ± 0.6	12.04 ± 0.65
Mean angular Displacement (m)	15.6 ± 1.7*	20.9 ± 1.6*	7.3 ± 1.3	6.21 ± 0.36
Lateral head displacement (μm)	2.3 ± 0.3	2.3 ± 0.1	1.0 ± 0.0	1.05 ± 0.04
Beat cross frequency (Hz)	1.6 ± 0.2*	1.3 ± 0.2*	0.7 ± 0.2**	0.17 ± 0.02**
Linearity (%)[1]	40.9 ± 1.8**	48.2 ± 1.3**	21.4 ± 1.0**	29.85 ± 1.16**
Wobble (%)[2]	56.0 ± 1.9**	63.4 ± 1.0**	29.8 ± 1.3**	41.00 ± 1.45**
Straightness (%)[3]	55.1 ± 2.1**	67.12 ± 1.1**	28.8 ± 1.4**	39.62 ± 1.58**
Viability (%)	76.7 ± 2.5*	82.4 ± 1.8*	59.4 ± 2.2	60.88 ± 1.64
Hypo-osmotic swelling test	61.9 ± 2.2*	68.7 ± 2.1*	43.3 ± 2.3	45.6 ± 1.8
Normal morphology	8.8 ± 0.6*	7.2 ± 0.4*	10.1 ± 0.7**	7.9 ± 0.5**
Sperm head (μm)	3.1 ± 0.3*	2.3 ± 0.2*	3.4 ± 0.3**	2.36 ± 0.2**
Sperm mid-piece length (μm)	1.2 ± 0.1**	0.8 ± 0.1**	1.1 ± 0.1**	0.8 ± 0.1**
Sperm tail (μm)	3.9 ± 0.4	3.6 ± 0.3	4.9 ± 0.5	4.2 ± 0.4

[1]Linearity:VSL/VCL, [2]Wobble: VAP/VCL, [3]Straightness: VSL/ VAP
Significant differences are marked with * (P < 0.05) and ** (P < 0.01)

Table 4. Significant multivariate effects in fresh and frozen thawed semen sample evaluated in different Straw type

Variables	Frozen thawed semen 0.25 ml Straw Mean ± S.E	Frozen thawed semen 0.5 ml Straw Mean ± S.E
Malondialdehyde (MDA)(nmol/ml)	71.3±2.5	75.2±1.2
Catalase (CAT) (nm/min/ml)	7.5±0.9	5.6±0.9
Superoxide dismutase (SOD)(Units/mg protein)	3.0±0.1	3.7±0.2

MDA levels were lower in 0.5 ml straws than 0.25 ml straws, but the differences were not statistically significant. CAT activity was higher in 0.5 ml straws than 0.25 one, but it was not statistically significant. Moreover, the differences between 0.5 ml and 0.25 straws were similar for SOD enzyme activity (Table 4).

Discussion

The purpose of our study was to compare bull semen quality, which is packed and frozen in two different sizes of straws (0.5 ml and 0.25 ml) and antioxidants and lipid peroxidation levels in frozen thawed semen. Although there are several studies which compare sperm motility parameters, viability, the hypo–osmotic swelling test (HOST), and acrosome integrity in some animals and humans, there are few studies which compare the morphology and oxidative enzymes activity as well as free radical levels of 0.5 ml and 0.25 ml straws in bulls. It has been suggested that reducing the surface to volume ratio in 0.5 ml straws reduces cryopreservation damage due to rapid sperm freezing (13). In contrast, Eriksson, et al. (15), claimed that semen packaging in a larger surface to volume ratio allowed for a more homogeneous freezing and thawing temperature and concluded that semen freezing in smaller volume packages could lead to less damage than those frozen in larger packages (20). It was demonstrated that the higher the surface to volume ratio in freezing, the greater the damage to bull sperm cells. However, they used some glass balls with three different diameters (70-110 µm, 250-500 µm, and 1,000-1,250 µm) in semen samples during freezing procedure and packed extended semen in either 8 ml or 2.5 ml cryogenic glass tubes. On the contrary, our findings showed that not only were the sperm motility parameters in 0.25 ml straws significantly higher than 0.5 ml straws, but also they had lower abnormal morphology percentage. The statistical analysis of abnormal spermatozoa frozen thawed semen demonstrated that the percentages of the total abnormal sperm, abnormalities of the head and mid piece of spermatozoa in 0.25 ml straws were significantly lower than the 0.5 ml straws.

Macpherson et al. (21) and Johnson et al. (12) reported that fertility results were not affected by the reduction of straw volume from 0.5 ml to 0.25 ml. In addition, they indicated that bull semen frozen in both 0.25 ml and 0.5 ml straw were similar in conception rate. On the other hand, a meta-analysis reported that there is not enough evidence to force all herdsman inseminators in the United States to use 0.25 instead of 0.5 ml straws, although there is a small outcome of pregnancy rate through insemination with 0.25 ml frozen thawed semen (10). Kang et al. (2020) suggested that freezing semen in 0.25 ml straw improves the relative motility, viability, and acrosomal enzymes, mitochondrial membrane potential, and plasma membrane integrity of Hanwoo bull spermatozoa in comparison with 0.5 ml straws. In buffalo, it was shown that freezing semen in 0.25 ml straws resulted in higher post thaw sperm quality compared to 0.5 ml straws (22) which is in line with our research findings. There is a report in which frozen thawed semen packed in 0.5 ml straws had higher gross motility, PM, VAP, VSL, VCL and rapid sperm than the 0.25 ml straws in stallions. In contrast, Consuegra et al. (8) reported that stallion sperm verification in 0.25 ml straws led to improved sperm quality after warming compared with conventional freezing. On the other hand, plasma membrane integrity evaluation did not show any significant difference. They concluded that stallion sperm frozen in 0.5 ml straws led to the improvements of semen quality after thawing in comparison with 0.25 ml straws (23), which do not support our results. This difference may be due to the differences between seminal components in these two different species. It has been shown that rainbow trout semen cryopreserved in 0.5 ml straws significantly improved the motility of sperm in thawed semen compared to a 0.5 ml ones (7). Furthermore, it was reported that the use of 0.5 ml straws led to higher or similar values of post-thaw sperm motility than the 0.25 ml straws in salmonid (4). In addition, it was reported that donkey sperm vitrification in 0.25 mL covered straw showed higher values than the 0.5 mL straw for total and progressive motility, plasma membrane and acrosome integrity (5). It was also reported that there was no significant difference between 0.5 ml straws and 0.25 ml straws in terms of sperm motility or viability in alpaca semen cryopreservation (2). Comparing the quality of human semen frozen in 0.25 and 0.5 ml straws, the researchers concluded that the percentages of motility, viability, mitochondrial membrane potential and intact chromatin of spermatozoa represented a higher rate in 0.5 ml straws rather than in 0.25 one. While the

percentage of sperms with intact acrosome was higher in 0.5 ml straws, the oxidative stress analysis did not show significant difference (24). On the other hand, it was shown that human semen frozen in a new micro straw with the volume of 100 µl had a significantly higher percentage of sperm motility than 0.25 ml and 0.5 ml straw (3). It was also reported that there were no significant differences in sperm viability, PM and HOST of semen frozen in either 0.25 ml or 0.5 ml straws in the boar, but the acrosomal normal apical ridge of 0.5 ml straws was significantly higher than that of 0.25 ml straws (25). In another study, it was nevertheless reported that boar semen frozen in 0.25 ml straws had more resistance against damage than sperms which were frozen in 0.5 ml straws (26). In the case of goat, it was also reported that PM and the acrosomal integrity of frozen thawed semen were significantly higher when semen was frozen in 0.5 ml rather than in 0.25 ml straws (27). In addition, in dogs it was shown that there were no significant differences in PM as well as abnormal acrosome of spermatozoa after semen thawing in both 0.25 and 0.5 ml straws (28). In a study conducted on cryopreservation of common carp semen, three different straw volumes (0.25, 0.5 and 1.5 ml) were compared, and it was suggested that the post thaw sperm motility and fertilization rate of semen packed in 1.5 ml straw was the best option for common carp semen freezing (29).

To the best of our knowledge there is no report regarding the comparison of enzymatic activity of frozen thawed semen in 0.25 and 0.5 ml straw in Simmental bulls. Our results showed that CAT activity was significantly higher in 0.5 ml straws than 0.25 one, but no significant difference was observed in the activity of SOD enzyme. Measuring MDA which is an indicator to determine the production levels of free radicals, we found that it was significantly lower in 0.5 ml straws than 0.25 ml straws. This may be due to the fact that the extender volume in 0.5 ml straws is twice as big as the size of 0.25 ml straws, which will reduce MDA levels as a result of more antioxidant contents in the Steridyl extender.

Our finding revealed that MDA (oxidative stress parameters) were more (not significant) in 0.25 ml straws than 0.5 straws, but it did not have detrimental effects on sperm parameters, although semen quality of 0.25 ml straws was better than that of 0.5 ml. As 0.25 ml straws had a higher surface to volume ratio rather than the 0.5 ml one, the temperature exchange between spermatozoa and nitrogen vapor was faster and provided good freezing and thawing. Lipid peroxidation could occur during freezing and thawing process in sperm. its seems that some stress such as cooling/osmotic are more important than ROS and LPO production on sperm quality and viability during freezing-thawing (17). figured out no differences between sperms from good freeze ability and poor freeze ability bulls for LPO(16).

The total antioxidant activity of sperm at frozen thawed semen as well as the superoxide dismutase (SOD) and catalase were observed without any significant changes between 0.5 and 0.25 ml semen straw. SOD and Catalase protected the sperm against oxidative stress (30). It seems that oxidative stress was the same in both 0.5 ml and 0.25 ml semen straw, although it was demonstrated that sod was not effective to prevent cryodamge in sperm to bull sperm (31).

Conclusion

According to the results of this study and other reports in bovines, it seems that sperm parameter quality after thawing of a 0.25 ml straw is better than 0.5 ml straw, or at least there is no significant difference between them. As a result, the cost of production in 0.5 ml straw is almost twice compared to 0.25 ml straws, which take more time to produce. According to the claims of several expert herdsman inseminators, the insemination semen in straws 0.5ml into one horn may allow some semen to back into the opposite uterine horn. Taking into account the economic conditions, we recommend that bull semen freezing centers produce frozen semen in 0.25 ml straws. In addition, promoting and creating the necessary culture for technicians and herdsman inseminators in this regard should be on the agenda of the officials of the National Breeding Centers of the countries.

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Conflicts of Interest

The authors of this manuscript declare no conflicts of interest related to this article

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