Reduced Glutathione and Cysteine Hydrochloride on Sperm Motility and Velocity Parameters of Poor Crossbred Bull Semen

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Article History

Manuscript No. 298
Received in 19th March, 2012
Received in revised form 29th March, 2012
Accepted in final form 5th June, 2012

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Keywords

Glutathione, cysteine hydrochloride, bull semen, velocity, motility

Abstract

The effect of reduced glutathione (5 mM) and cysteine hydrochloride (5 mM) on sperm velocity and motility parameters of good (3) and poor (3) freezable Jersey crossbred bull semen was studied. The post-thawed sperm velocity and motility parameters were assessed by computer assisted sperm analyzer (CASA) at different hours of incubation. The forward progressive and total motility showed significant ($p<0.05$) difference in glutathione treated group as compared to cysteine treated and control group in both freezer. In both freezers, the forward progressive and total motility was not decreased significantly up to 2 h of incubation but thereafter there was a highly significant decrease. A gradual non-significant decline in all the velocity parameters in good freezable semen samples were observed for the 4 periods of incubation and in poor freezable semen, the reduction in the velocity parameters was not significant up to 2 h of incubation, but thereafter, the decrease was significant. The velocity parameters of sperm revealed that the glutathione treated group was non-significantly higher than cysteine treated and control group in both good and poor freezable bulls except the addition of glutathione significantly improved VCL (curvilinear velocity) and ALH (amplitude of lateral head displacement) of the post-thawed spermatozoa at 4 h of incubation. Further, the addition of cysteine and glutathione has non-significantly improved the other velocity parameters in both the freezer. Thus, the reduced glutathione has improved the post-thawed sperm functional parameters such as motility and velocity in good and maintained in poor freezable semen.

1. Introduction

Artificial insemination has made a profound contribution to the genetic improvement, particularly in dairy bulls through which a single ejaculate from a male is used to impregnate many females. The process of cryopreservation exerts physiological, osmotic and chemical stress on the sperm membrane and sperm structure, which may result into the damage of spermatozoa and post-thawed quality of spermatozoa (Ozkavukcu et al., 2008). The spermatozoa membrane has highly unsaturated fatty acid content (Sinha et al., 1996). Lipid peroxidation of spermatozoa membrane eventually leads to apoptotic cell changes and loss of sperm function (Sikka, 1996) in post-thawed semen samples. The cryopreservation reduces the content of reduced glutathione of spermatozoa and seminal plasma (Gadea et al., 2008) and leads to changes in membrane transportation (Alvarez and Storey, 1982) in turn affecting fertility of the spermatozoa. The fact that males can often be classified as good freezers or bad freezers implies that certain characteristics of membrane structure, which may be genetically determined, predispose towards survival under cryopreservation stress (Watson, 2000). There are instances in which certain semen samples within a species having good pre-freeze motility, still results in poor freezability. Differences in either ejaculation frequency, or in epididymal transit times and sperm mixing in the epididymis, provide a potential mechanism for variability in responses to subsequent temperature explaining why ejaculates within individuals can vary in their responses to cryopreservation (Watson, 1995). Sperm motility in general and characteristics of sperm motion in particular could be some of the indicators of the quality of spermatozoa. Commonly, by evaluating the proportion of progressive motile percent at different stages, the quality of semen is monitored. However, evaluation

of characteristics of sperm motion may provide valuable information on why certain samples despite containing good proportion of progressive motile spermatozoa pre-freezing poorly freezable. Computer assisted sperm analyzer (CASA) technique was used to provide precise and accurate information on sperm motion characteristics. Therefore, a study was undertaken to analyze the changes in motility characteristics of Jersey crossbred spermatozoa during thawing (35°C) at different hours of incubation by CASA technique and its relevance in post-thawed survival of spermatozoa. Perusal of literature revealed paucity of information on the ability of reduced glutathione in improving freezability of poor freezable bull. Hence, the objective of this study was to assess the effect of reduced glutathione in different motility and velocity parameters of sperm by CASA to improve the semen quality in good and poor freezable Jersey crossbred bull semen.

2. Materials and Methods

The experiment was conducted in 3 good freezable and 3 poor freezable Jersey crossbred bulls belonging to Frozen Semen Bank, Cuttack, under Animal Husbandry and Veterinary Services, Government of Odisha, India. The experimental bulls of 4-6 years of age with good body condition (score 5-6) were selected and maintained under optimum managemental practices as per the standard criteria fixed for maintenance of breeding bulls in bull stations. The pre-freezing seminal parameters of both groups of semen had >60% progressive forward motility, less than 20% total abnormality. The good and poor freezable bulls were classified based on post-thawed motility parameter. Those bulls provided consistently more than 40% post-thawed motility were considered as good freezable bulls (n=3), whereas those bulls with less than 40% post-thawed motility were considered as poor freezable bulls (n=3). Ejaculates (6) from each bull were collected twice in a week. Each ejaculate was split in to 3 equal parts to use one part as control without any additives; second and third part was added with cysteine hydrochloride and reduced glutathione @ 5 mM, respectively for cryopreservation and subsequent evaluation. All experimental semen samples were extended with Tris extender and frozen by rapid freezing method in mini straws and cryopreserved using programmable freezer and stored in liquid nitrogen. Analysis of the motion parameters were determined using a CASA system (Sperm Class Analyzer, Microptic, Barcelona, Spain) at 0, 1, 2 and 4 h following the thawing of semen sample. Analysis was described by Gadea et al. (2008) and was based on the examination of twenty-five consecutive digitalized images second^{-1} using a 10X negative phase contrast objective (Nikon, Eclipse E200) and fitted with stage warmer (Linkam DC 60). The CASA-derived motility characteristics studied were total motility (%), progressive motility (%), non-progressive motility (%), static sperms (%), curvilinear velocity (VCL in μm s^{-1} is the average path velocity of the spermatozoa head along its actual trajectory), straight-line velocity (VSL in μm s^{-1} is the average path velocity of the spermatozoa head along a straight line from its first to last position), average path velocity (VAP μm s^{-1}), linearity of the curvilinear trajectory (LIN is the ratio between VSL and VCL in %), straightness (STR, ratio of VSL/VAP in %), wobble of the curvilinear trajectory (WOB, ratio of VAP/VCL in %), amplitude of lateral head displacement (ALH in μm s^{-1} is the average value of the extreme side-to-side movement of the spermatozoa head in each beat cycle) and beat cross-frequency (BCF, Hz). The parameters set were frame rate: 60 Hz frame acquired: 25, minimum contrast: 30, cell size (range 5 to 70 micron^2), spermatozoa concentration (5 to 8 x 10^6 ml^{-1}), and motile cells (>10 μm s^{-1} at 37°C), progressive forward motility (>80% straightness index, STR); circular movement (<50% linearity index, LIN). Motility analysis was carried out in 5 μl of semen samples (0.25 ml diluted semen in 1.75 ml of Tris buffer) placed onto a pre-warmed (37°C) microscopic slide covered with the 18x18 mm^2 cover slip. The results were analyzed statistically after arcsine transformation of percentage data by using SPSS 15 (SPSS, Chicago, IL, USA).

3. Results and Discussion

The post-thawed sperm forward progressive motility significantly (p<0.05) differed between the good and poor freezable bull at different time periods (Table 1).

The non-progressive motility was significantly higher between the freezers at 1 h of incubation, whereas the total motility and static sperms were significantly (p<0.05) different at 4 h of incubation. Both in good and poor freezable semen, the lowering of proportion of total sperm motility and progressive sperm motility during different periods of incubation were highly significant. However, in both freezers, the decrease in forward progressive and total motility was not significant up to 2 h of incubation but was highly significant thereafter (Table 1). A gradual non-significant decline in all the velocity parameters, namely average path velocity (VAP) and curvilinear velocity (VCL) in good freezable semen sample were observed for the 4 periods of incubation. Whereas, in poor freezable semen samples, the reduction in the velocity parameters was not significant only up to 2 h of incubation, but the decrease was significant thereafter for VAP and VCL. For other motility characteristics the variation exhibited was not significant (Table 2).

Freezing had a significant effect on total motility, progressive motility, VAP, progressive velocity, VCL, beat cross frequency, straightness, linearity and lateral amplitude of head of bovine spermatozoa. The good freezable semen samples was recorded a highly significant decrease in total motility and progressive motility and also a significant reduction in all the velocity

Table 1: Assessment of motility parameters of good and poor freezable crossbred bull semen by computer assisted sperm analyzer (CASA)

Parameters \ Bull	Good freezer (18)	Poor freezer (18)
0 h		
Forward progressive motility (%)	67.07±1.67[a]	56.71±2.42[b]
Non-progressive motility (%)	17.74±1.89	21.70±21.50
Total motility (%)	84.80±1.87	78.41±2.75
Static sperms (%)	15.20 ±1.87	21.59±2.751
1 h		
Forward progressive motility (%)	69.02±3.57[a]	49.42 ±3.51[b]
Non-progressive motility (%)	13.97±2.47[a]	25.25±3.11[b]
Total motility (%)	83.00±2.18	74.67±5.39
Static sperms (%)	17.00±2.18	25.33±5.39
2 h		
Forward progressive motility (%)	54.13±3.72[a]	43.88±2.62[b]
Non-progressive motility (%)	15.33±2.64	21.87±2.97
Total motility (%)	69.45±3.12	65.74±3.62
Static sperms (%)	30.55±3.12	34.26±3.62
4 h		
Forward progressive motility (%)	42.84 ±4.30[a]	28.55±3.63[b]
Non-progressive motility (%)	18.09±2.69	21.51±2.37
Total motility (%)	60.93±3.53[a]	50.05±3.80[b]
Static sperms (%)	39.07±3.53[a]	49.95±3.80[b]

Means with different superscripts in rows varied significantly in different time periods ($p<0.05$); Figures in the parenthesis indicate number of ejaculates

parameters and beat cross frequency gradually from 0 to 4 h of incubation. In contrast, semen with poor freezability had shown a highly significant decline, not only in total motility and progressive motility, but also other parameters like the three velocity parameters and linearity besides a significant reduction in beat cross frequency and straightness (Table 2). The forward progressive motility and total motility was higher in good freezer as compared to poor freezer in different treatment groups and glutathione treated group had higher forward progressive and total motility than the cysteine hydrochloride treated and control group in different time of incubation period (Table 2). Similarly, non-progressive and static sperm percentage was higher in poor freezer as compared to good freezer and was higher in control and cysteine hydrochloride treated groups as compared to glutathione added group in different time of incubation period (Table 3), which is very comparable to the earlier findings (Uysal et al., 2007).

However, slightly lower motility was reported in glutathione added semen in crossbred bulls (Munsi et al., 2007). Similarly, reduced glutathione added group showed non-significantly higher value as compared to cysteine and control group in different time periods of incubation from 0-4 h (Table 3). The reduced glutathione treated sperm had more and fast forward movement as compared to control group because the reduced glutathione neutralizes and prevented the formation of free radical and maintains the membrane integrity and increased the efficiency of flagellar motion efficiently in fluid medium. Moreover, glutathione also improved the efficiency of mitochondria (Perumal et al., 2011ab) and also protected the other parts of sperm membrane to maintain the motility (Gonçalves et al., 2010). During freezing, the spermatozoa membrane is damaged resulting in leakage of enzymes. The present study revealed that addition of glutathione in freezing extender prevented leakage of enzymes through the action of membrane stabilizer as stated by Maxwell and Stojnov (1996). The evaluation of reduced glutathione in good and poor freezable semen samples in both classes showed similar trends for changes in total motility and progressive motility besides changes in certain other motility characteristics namely, lateral amplitude of head, beat cross frequency, straightness and linearity. However the decrease in speed of spermatozoa in terms of VAP and VCL was significant only in samples that froze poorly. Furthermore, on comparing the movement characteristics of spermatozoa between good and poor freezable semen samples during incubation, it was found that the progressive speed of spermatozoa in the inferior samples was significantly lower than that of the samples that froze satisfactorily following 3 and 4 h of incubation (Table 1). However, the addition of glutathione significantly improved VCL and ALH of the post-thawed spermatozoa. Further, the addition of cysteine and glutathione non-significantly improved VSL and VAP. Spermatozoa kinematic variables such as progressive forward motility, VSL, VCL, ALH and LIN were correlated to bull fertility (Al-Qarawi et al., 2002). In the glutathione treated group, a significantly higher VCL and ALH of the spermatozoa indicated major bending of the mid piece and large amplitude of ALH (Table 2). This signified that glutathione induces hyperactivation of the spermatozoa. Hyperactivation in turn implied high energy state of the spermatozoa, which is essential for sperm penetration

Table 2: Assessment of velocity parameters of sperm by computer assisted sperm analyzer (CASA)

Parameter	Group: I (6)		Group: II (6)		Group: III (6)	
	Good	Poor	Good	Poor	Good	Poor
0 h						
VCL	125.03±4.33	111.36±12.78	112.75±4.42	110.08±10.30	130.76±3.47	138.63±4.1
VSL	93.43±3.21	79.72±11.60	76.20±10.37	73.04±8.29	91.84±3.39	87.23±11.03
VAP	111.66±5.12	95.65±11.46	89.93±10.12	86.28±7.14	111.47±5.4	103.42±12.5
LIN	74.83±2.58	71.11±2.11	67.72±9.10	66.20±2.38	66.21±7.01	66.22±0.58
STR	84.09±3.14	82.87±2.06	84.28±2.15	84.24±2.48	83.75±1.67	82.50±1.72
WOB	89.05±1.16	85.79±0.99	79.96±8.92	78.64±2.81	80.32±1.70	78.64±7.86
ALH	2.88±0.03	2.84±0.27	4.01±0.17	3.11±0.40	4.00± 0.16	3.59±0.24
BCF	9.77±0.49	9.54±0.82	10.48±1.37	11.92±0.30	11.52±1.22	11.43±0.65
1 h						
VCL	105.82±12.47	93.81±7.27	112.59±12.08	94.25±9.89	102.39±5.26	96.02±20.4
VSL	59.14±5.88	56.73±7.16	66.16±4.49	60.22±11.62	64.20±10.94	57.09±13.9
VAP	75.24±8.07	69.15±6.08	83.37±7.55	68.42±10.38	74.10±8.82	70.59±15.2
LIN	62.87±3.24	53.74±4.49	63.01±7.39	59.63±4.83	62.87±10.18	58.60±3.17
STR	85.11±1.70	75.11±3.31	86.61±3.52	79.84±3.58	85.32±5.26	79.79±3.87
WOB	73.73±2.20	71.31±2.83	72.19±5.86	74.46±3.13	72.53±8.06	73.40±0.44
ALH	3.36±0.39	2.76±0.28	3.47±0.33	2.75±0.16	2.87±0.22	2.83±0.44
BCF	14.06±0.15	13.19±0.61	14.25±0.78	14.28±1.05	14.56±0.131	14.54±0.31
2 h						
VCL	103.82±9.25	92.78±3.90	100.59±4.42	96.93±18.81	125.10±3.83	107.21±12.23
VSL	64.28±5.19	56.42±1.94	61.02±5.12	54.49±12.43	77.44±2.43	70.08±17.32
VAP	79.12±6.28	69.30±3.41	73.83±4.16	68.02±14.57	96.51±0.75	81.43±15.43
LIN	61.00±1.06	62.39±5.34	60.81±4.05	55.82±8.13	63.94±9.44	62.04±3.07
STR	81.47±1.46	80.21±1.89	82.52±2.99	79.56±5.58	84.76±6.22	81.38±3.94
WOB	77.74±0.69	76.40±2.76	73.51±2.23	69.48±5.24	77.30±2.58	74.89±5.91
ALH	2.68±0.20	2.84±0.19	2.92±0.24	3.13±0.46	2.85±0.16	3.56±0.038
BCF	14.68±0.74	14.56±0.10	14.90±0.21	13.14±0.99	14.94±0.81	14.88±0.11
4 h						
VCL	104.96±11.96	98.89±1.20	106.11±5.89	100.59±4.42	120.37±9.23	118.13±14.46
VSL	52.60±9.04	49.70±12.04	61.02±5.12	50.69±9.80	56.72±10.45	58.66±10.54
VAP	69.29±5.02	68.20±13.01	73.83±4.16	68.10±11.61	83.18±13.71	77.59±9.36
LIN	48.51±8.47	46.77±9.33	60.81±4.05	47.18±7.02	53.37±8.64	48.63±6.80
STR	72.64±6.55	70.44±5.29	82.52±2.99	74.03±2.52	76.34±7.02	71.82±5.50
WOB	69.46±4.96	64.09±7.98	73.51±2.23	63.42±7.82	65.75±6.26	68.54±5.23
ALH	3.55±0.33	3.09±0.23	3.92±0.24	3.92±0.30	3.84±0.18	3.30±0.18
BCF	13.54±1.07	11.95±0.31	14.90±0.21	11.68±0.47	13.88±0.44	13.95±0.97

VCL: Curvilinear velocity (μm s^{-1}); VSL: Straight line velocity (μm s^{-1}); VAP: Average path velocity (μm s^{-1}); LIN: Linearity (%); STR: Straightness (%); WOB: Wobble (%); ALH: Amplitude of lateral head displacement (μm); BCF: Beat/cross frequency (Hz); Figures in the parenthesis indicate number of ejaculates

Table 3: Motility parameters of good and poor freezable crossbred bull semen between different experiment groups by computer assisted sperm analyzer (CASA)

Parameter	Group: I (6)		Group: II (6)		Group: III (6)	
	Good	Poor	Good	Poor	Good	Poor
0 h						
Progressive forward motility	69.17±4.96	51.03±7.09	69.76±3.23^{a}	55.04±4.76^{b}	71.47±8.40^{a}	53.10±3.99^{b}
Non-progressive motility	13.97±2.08	22.95±7.36	16.83±2.08	21.63±5.59	13.89±7.11	31.10±4.98
Total motility	83.14±5.90	73.98±9.09	86.59±2.82^{a}	76.66±2.35^{b}	85.36±1.62	84.20±3.50
Static sperms	16.86±5.90	26.02±9.09	13.41±2.82^{a}	23.34±2.35^{b}	14.64±1.62	15.80±3.50
1 h						
Progressive forward motility	65.38±4.04	57.08±5.32	66.43±7.21^{a}	44.14±7.75^{b}	66.05±1.05	58.02±4.13
Non-progressive motility	18.31±5.05	21.42±1.90	14.05±4.32	21.70±5.99	18.07±3.54	22.05±4.52
Total motility	83.69±3.33	78.50±4.34	80.49±3.70^{a}	65.83±1.30^{b}	84.13±4.53	80.06±7.97
Static sperms	16.31±3.33	21.50±4.34	19.51±3.70^{a}	34.17±1.30^{b}	15.87±4.53	19.94±7.97
2 h						
Progressive forward motility	52.86±6.20	43.77±4.73	49.13±7.14	46.63±4.25	60.38±6.62^{a}	41.24±5.90^{b}
Non-progressive motility	17.96±3.89	17.61±1.50	13.83±2.12	24.00±8.70	14.19±7.62	23.99±3.75
Total motility	70.82±2.62	61.38±4.37	62.96±8.62	70.62±1.05	74.57±1.06^{a}	65.23±2.50^{b}
Static sperms	29.18±2.62^{b}	38.62±4.37^{a}	37.04±8.62	29.38±1.05	25.43±1.06	34.77±2.59
4 h						
Progressive forward motility	39.58±1.06^{a}	25.09±3.99^{b}	42.96±5.60^{a}	29.77±3.69^{b}	46.98±8.87^{a}	30.79±1.09^{b}
Non-progressive motility	21.52±4.35	22.70±4.87	14.71±6.52	21.35±3.96	18.04±3.92	20.48±5.20
Total motility	61.10±6.25^{a}	47.49±1.03^{b}	57.67±6.93	51.11±5.65	64.02±7.25	49.56±4.31
Static sperms	42.33±6.93	46.51±1.03	38.90±6.25	48.89±5.65	35.98±7.25^{a}	54.44±4.31^{b}

Means with different superscripts in a row varied significantly within a treatment group (p<0.05); Figures in the parenthesis indicate number of ejaculates

through cervical mucus and fuse with the oocytes (Mortimer, 1994). It had been established that the assessment of sperm motility in a semen sample might not be a reliable index for semen evaluation (Giritharan et al., 2005). The objective and quantitative measurement of other sperm motion characteristics of individual cells assessed by CASA was found to be more efficient in predicting semen fertility potential (Mortimer, 1994). In bovine, specific motion parameters reported to be related to fertility (Farrell et al., 1996). The study suggested that the glutathione might positively influence fertility by influencing VCL and ALH of the spermatozoa in both good and poor freezer. Even among good and poor freezable samples there was not much of difference in sperm behavior due to freezing and thawing. Good freezable reduced glutathione added group showed higher motility and velocity parameters value than poor freezable glutathione added semen, is due to the response towards the effects glutathione on good freezable is higher (Table 2). The main effect of glutathione on good freezer was to prevent change the osmotic pressure during semen processing for cryopreservation, which critically affected the spermatozoa. This may be the most important deterrent to sperm survival during cryopreservation (Watson, 1995). Furthermore, it prevented membrane destabilization of the sperm plasma membrane, which stopped phase transition from the liquid crystalline phase to the gel phase due to a decrease in temperature (Barrea-Compean et al., 2005). Thus it prevented the irreversible changes in the sperm membrane induced by lipid phase transitions during cooling warming might possibly affect the movement characteristics of spermatozoa during semen processing for cryopreservation (Holt and North, 1984; Deleeuw et al., 1990). In addition, frozen-thawed sperm was more vulnerable to oxidative stress due to peroxidation than sperm in freshly diluted semen (Neild et al., 2005). As semen was diluted many fold in the extender it reduced the total antioxidant concentration in the medium and cells (Kumar and Das, 2005). Many sperm were killed during cryopreservation. Thus, it is likely that cryopreserved sperm cells were posed to more ROS concentration and therefore many of the surviving cells

post-thawed exhibited as if they were capacitated or acrosome reacted (Bailey et al., 2000). The overall effects of these events might adversely affect quality of post-thawed semen. But glutathione added semen prevented all these adverse effects of ROS in semen preservation which was higher in good than poor freezable semen because response towards the former than latter. The reason behind is not known. In the present study, the glutathione supplementation maintained membrane integrity as well as post-thawed motility of the spermatozoa. Stabilization of the spermatozoa1 plasma lemma by glutathione might have protected the acrosomal enzymes during freezing and thawing. This could be the reason for improved conception rate in glutathione treated group (Figure 1). Further, the reduced glutathione enhanced the fertility parameters in good

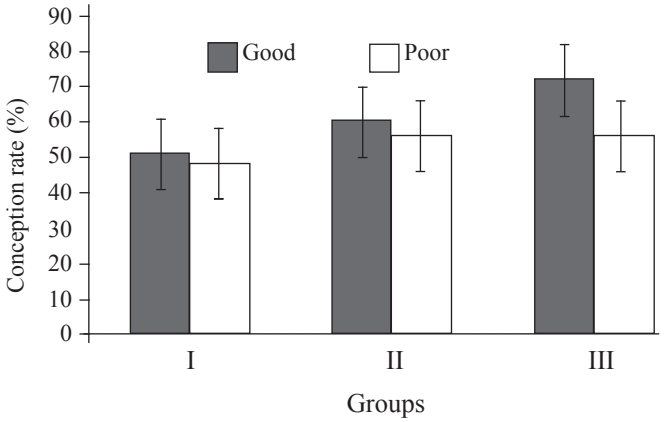

Figure 1: Conception rate of good and poor Jersey crossbred bulls with different semen additives

freezer and maintained in poor freezer.

4. Conclusion

The present study provides evidence that the use of glutathione as semen additives may be recommended for improving semen quality and overall augmentation of pregnancy in cows in good and poor freezable bulls.

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