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Research Article

Effect of Various Concentration of Glycerol on Post Thaw Quality of Holstein-Friesian Bull Semen

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Abstract

The aims of study were to compare the glycerol concentration in Tris, Citric acid Egg yolk diluents on the quality of Holstein Friesian frozen semen. Three Holstein Friesian bulls were maintained to collect the 45 semen samples. Total of 15 ejaculates sample were collected from each bull. Fresh semen was evaluated for volume, color, pH, concentration and motility. Every collection of 3 ejaculate was pooled and examined. Each pooled semen sample was divided in to three experimental extenders A, B, C, glycerol was used as diluents at 7, 6, 5%, Pencillin (1000 I.U/ml) and streptomycin (1000g/ml) were added to each diluent. Immediately after completion of the semen extension process, the post dilution motility percentage of each extender was noted. After 24 hours of freezing and storage liquid nitrogen -196 ^{o}C, three sample straws from each glycerol concentration were taken out and thawed in water bath at 37^{0}C for 15 seconds. After thawing the semen was tested by sperm quality analyzer SQA vb for bull post thaw motility % , progressive motility %, average pathway velocity µm/s, straight-line velocity µm/s, curvilinear velocity µm/s varied significantly between 5, 6 and 7% glycerol concentration to treatment $p>0.05$. Results of the experiment demonstrated that post thawed sperm motility in the semen preserved with 7% glycerol higher than other levels of glycerol, PTM (49.64%), PM (34.76%), VAP (94.92 um/s), VSL(85.36 um/s), VCL(165.42 um/s). It was concluded that 7% glycerol is recommended for freezing media regarding with best result on post-thaw quality of Holsteins-Friesian bull semen.

Keywords: Semen, Glycerol, Post thaw quality, Holstein Frisian

Introduction

Over the past 60 years, the cryo-protective media for sperm storage have been continuously revised, but the basic ingredients of the media remain unchanged. Glycerol

and egg yolk represent the indispensable compounds of practically all media used for bull sperm preservation in liquid or frozen states. It is also clear that the interaction between the sperm and surrounding medium is a crucial factor affecting the preservation of sperm integrity and fertilizing ability (Bindari *et al.*, 2013). Successful cryopreservation depends on the survival of cellular components, including the sperm, plasma membrane, acrosome, and nucleus. If the cellular components are damaged or destroyed during the freeze-thaw process, the spermatozoa are rendered useless for fertilization if used for artificial insemination (AI) (Nair *et al.*, 2006). Glycerol is a colorless and odorless liquid that is sweet in taste, a universal preservative and lubricant, and widely used in the pharmaceutical industry (Kaka *et al.*, 2012). Freezing and thawing stresses can be minimized by the use of glycerol (4–8%), which is an osmotically active permeating cryoprotectant. The physiological actions of glycerol in freezing and thawing spermatozoa take place by replacing intracellular water necessary for the maintenance of cellular volume, interaction with ions and macromolecules, and depressing the freezing point of water so that less ice forms at any given temperature (Jamali *et al.*, 2019; Medeiros *et al.*, 2002). Glycerol, though, has also been shown to have some adverse effects on spermatozoa due to osmotic stress, changes in membrane organization, fluidity, and permeability, as well as changes in the lipid composition (Watson, 1995). Keeping in view the factors regarding the concentration of glycerol in the extender for deep freezing of the bull semen, it is considered necessary to further investigate the effect of various concentrations of glycerol (7%, 6%, and 5%) with the Tris, citric acid, fructose, and egg yolk extender on the various quality characteristics of frozen Holstein-Friesian bull semen. This study will also help to develop and formulate a more precise and optimal extender for the preservation of bull semen.

Methodology

The Semen Production Unit, Brewary Road, Quetta, Livestock and Dairy Development, Government of Balochistan, maintained the influence of various glycerol concentrations on the post-thaw quality of Holstein-Friesian bull semen throughout the months of April and May 2012. The experimental details from Ha *et al.* (2012) study, which involved frozen semen producing bulls, were applied to these breeding bulls in the same manner.

Collection of semen

Before the semen was taken, the bulls were properly cleaned and shaved. Prior to the collection of semen, all hygienic precautions were done. Two false mounts and five to ten minutes of sexual restraint were used to excite the bulls' sex. Three Holstein Friesian bulls were kept to collect the 45 semen samples, and 15 ejaculates were taken from each bull. Artificial vagina was employed for semen collection, and all normal procedures were followed.

Evaluation of semen

Following semen collection, samples were immediately placed in a water bath set at 37° C for 15 minutes. The semen's volume, color, pH, motility, and sperm concentration were also assessed during this procedure utilizing a Sperm Quality Analyzer (SQA).

Color

Color was observed macroscopically as creamy and white Patel and Siddiquee (2013).

pH

pH was determined by using digital pH meter.

Volume

Semen volume (v) was observed through reading the different graduation marking as suggested by Ha *et al.* (2012).

Motility

The subjective motility was assessed with the phase contrast microscope (×400) connected with a closed circuit television. Only semen samples with adequate motility more than 70 % were selected for further processing (dilution, cooling, equilibration and freezing).

Sperm Concentration

Sperm concentration was observed using the hemocytometer as suggested by Kaka *et al.* (2012).

Thawing of Semen Straws and Evaluation

After 24 hours of freezing semen from straws in liquid nitrogen, three straws from each glycerol concentration were taken out and thawed at 37 ℃ for 15 seconds. Suggested by Kaka *et al.* (2012), evaluate three thawing rates for bull semen frozen in 0.5-ml straws: placing the straws in a water bath at 37 degrees C for 40 seconds, at 50 degrees C for 15 seconds, or at 70 ℃ for 5 seconds. Experimental parameters motility, progressiveness, and velocity were checked using SQA (Sperm Quality Analyzer Vb Version 1.01 S No. 1023, made in America). Each sample of 20 µL aspirated into the disposable capillary was inserted into SQA-Vb. Measurements were displayed within 75 seconds. Kaka *et al.* (2012) reported that computer-assisted sperm analysis (CASA) was used to determine total sperm motility (TM), progressive motility (PM), average path velocity (VAP), straight line velocity (VSL), and curvilinear velocity (VCL).

Data analysis

Data were analysed using the statistical program computer package "statistix 8.1". Analysis of variance (ANOVA) was used to test for significant differences in semen volume, semen concentration, semen pH, motility, progressive motility and velocity.

Results and Discussion

The study was carried out to evaluate the effect of different levels of glycerol on post thaw quality of bull semen of Holstein- Friesian bull semen. A total 45 ejaculate were taken from three different bulls. Fifteen ejaculates from each bull were taken.

Mean of volume, pH, concentration and fresh motility % of Holstein Friesian bull semen

Mean of volume, pH, concentration and fresh motility % of Holstein Friesian bull semen showed highly significant response of treatment at P<0.05 (Table-1). The maximum mean value was recorded in bull C followed by bull B. While the minimum semen volume mean was recorded in case of bull A.

Table 1. Mean of Volume pH, concentration and fresh motility % of Holstein Friesian bull semen.

Variable	Volume	pH	Concentration	Fresh motility %
Bull A	4.5	6.6	1240.7	79.33
Bull B	5.1	6.7	1199.0	75.00
Bull C	5.5	6.8	1228.0	76.66

LSD>0.05 0.4379

Mean of post dilution motility of Holstein Friesian bull semen

Mean of post dilution motility of Holstein Friesian bull semen showed highly significant response of treatment at P<0.05 (Table-2). The maximum post dilution motility mean value was recorded in glycerol 7% followed by bull C and bull B. While the minimum post dilution motility mean was recorded in case of glycerol 5% in all Bulls.

Table 2. Mean of post dilution motility of Holstein Friesian bull semen.

Variable	Glycerol		
	7%	6%	5%
Bull A	72.66	70.67	68.73
Bull B	71.48	69.55	67.23
Bull C	74.92	73.85	70.52

LSD > 0.05 2.6018

Mean of post thaw motility of Holstein Friesian bull semen

Mean of post thaw motility of Holstein Friesian bull semen showed highly significant response of treatment at P<0.05 (Table 3). The maximum post thaw motility mean value was recorded in glycerol 7% followed by bull C and bull B. While the minimum post dilution motility mean was recorded in case of glycerol 5% in all Bulls.

Table 3. Mean of post dilution motility of Holstein Friesian bull semen.

Variable	Glycerol		
	7%	6%	5%
Bull A	49.64	47.51	45.53
Bull B	47.42	45.23	43.17
Bull C	49.16	46.64	44.64

LSD> 0.5 1.4766

Mean of progressive motility of Holstein Friesian bull semen bull

Mean of progressive motility of Holstein Friesian bull semen showed highly significant response of treatment at P<0.05 (Table 4). The maximum progressive motility mean was recorded in glycerol 7% from bull C followed by bull B and bull. While the minimum progressive motility mean was recorded in glycerol 5% from all bull.

Mean of Average Path Velocity micron per second of Holstein Friesian bull semen

Mean of VAP of Holstein Friesian bull semen showed highly significant response of treatment at P<0.05 (Table 5). The maximum VAP mean was recorded in glycerol 7% from bull C followed by bull B. While the minimum VAP mean was recorded in glycerol 5% from all bulls.

Table 4. Mean of progressive motility of Holstein Friesian bull semen.

Variable	Glycerol		
	7%	6%	5%
Bull A	34.06	32.17	32.48
Bull B	33.33	31.35	29.82
Bull C	34.76	32.94	31.92

LSD> 0.05 1.2490

Table 5. Mean of VAP micron per second of Holstein Friesian bull semen.

Variable	Glycerol		
	7%	6%	5%
Bull A	92.92	89.66	87.22
Bull B	93.81	90.82	89.10
Bull C	94.67	91.85	89.16

LSD> 0.05 1.3708

Mean of straight line velocity micron per second of Holstein Friesian bull semen

Mean of straight line velocity of Holstein Friesian bull semen showed highly significant response of treatment at P<0.05 (Table 6). The maximum straight line velocity mean was recorded in glycerol 7% from bull C by bull B. While the minimum straight line velocity mean was recorded in glycerol 5% from all bulls.

Table 6. Mean of VSL micron per second of Holstein Friesian bull semen.

Variable	Glycerol		
	7%	6%	5%
Bull A	83.36	80.80	78.24
Bull B	8475	81.50	79.79
Bull C	85.35	82.92	80.39

LSD> 0.05 1.1248

The findings of the current study showed that the ejaculate volume from Holstein Friesian bulls is significantly varied between the means. Bull C had the highest mean ejaculate volume at 5.5 ml, followed by Bull B at 5.1 ml, while Bull A had the lowest mean at 4.5 ml. Overall, these results are consistent with the work of Ha *et al.* (2012), who reported a range of 4.63 to 8.01 ml for the volume of semen. According to Angasaria *et al.* (2002), the average ejaculatory volume of semen for two HF bulls was 4.70 0.159 ml and 4.76 0.167 ml, respectively. Similar to this, mean values of 4.04 0.03 and 2.92 0.03 ml are relatively lower. The result achieved from the present study that the ejaculation concentration of the sperms collected from Holstein Friesian Bull semen were highly significant. Patel and Siddiquee (2013) showed the same result of sperm concentration. Ha *et al.* (2012), reported that sperm concentration fluctuated between 1.06 to 1.34 billion/ml Holstein Friesian bull lower than 2541.9 ± 1699.2 million/ml for indigenous non-descript zebu bulls in India recorded by Siddiqui *et al.* (2008). The differences in semen parameters among bulls may be due to variations in secretary activities of the sex glands, scrotal circumference, breed, age, body size and body weight (Graham, 1996). The result showed from the present research work that the ejaculate fresh motility from Holstein Friesian Bull was significantly importance. The maximum fresh motility mean achieved from Bull A 79.33% followed by Bull C 76.66% while the minimum fresh motility of semen was achieved from Bull B 75 %. Similar result with Ardelean *et al.* (2013) mobility average values of fresh semen was 68.33±1.55% in bull B and 72.50±0.75% in bull A. The overall mean sperm motility in the present study was 74.13%, being highest for the bull A i.e. 78.00% and 67.00% being lowest for the bull C 70.13%. These

results are consistent with those of Angasaria *et al.* (2002), who reported that initial motility ranged from 30 to 83% with averages of 67.24 to 2.59 and 66.80 to 2.28, respectively. Ha *et al.* (2012) and Siddiqui *et al.* (2008) also reported lower sperm motility of 50.8 to 17.2% for native nondescript zebu bulls in India. Similar with the result of Taşdemir *et al.* (2013) 6% Glycerol exhibited the greatest percentages of CASA (43.7±2.92%) and progressive (26.4±2.64%). According to Spaleková *et al.* (2013), the range of PM values after thawing was between 29.37% (RAD 229) to 60.5% (BA 100).According to Thérien and Manjunath (2003), there are differences between glycerol and DMF in terms of both subjective and objective progressive motility (43.1% vs. 21.5% and 11.8% vs. 6.2%, respectively). The Holstein Friesian bull's mean VAP micron per second exhibited a highly significant response to treatment (P 0.05). In glycerol 7%, bull A recorded the highest mean velocity at 94.92 m/s, followed by bull C at 91.66 m/s and bull B at 89.22 m/s. According to Ardelean *et al.* (2013), the velocity parameter, which measures the percentage of sperm cells with average path velocity (VAP), has rather stable values (between 71.032.13% in bull F and 95.524.26% in bull C). All 6 bulls had an average sperm cell velocity recorded after thawing of 81.078.94 m/s. Jamali *et al.* (2019) reported a percentage of progressive spermatozoa, path 72.161.85 (VAP) and progressive 58.652.23 (VSL) velocity. These finding are similar with Jamali *et al.* (2019) VSL = 70, 75 and 89 µm/s vs 59 µm/s. Mean of velocity micron per second of Holstein Friesian bull semen showed highly significant response of treatment. The maximum VCL was recorded in glycerol 7% followed by bull A 162.89 µm/s followed bull C 162.89 µm/s bull B 161.25.While the minimum velocity mean was recorded in case of glycerol 5% 156.27 micron/sec respectively in present study, similarly reported Najjar *et al.* (2013) VCL = 132, 138 and 187 µm/s vs 116 µm/s.

Conclusion

It is concluded from the research work that the ejaculate fresh motility, pH, volume, concentration, post dilution motility, post thaw motility, progressive motility and velocity varied significantly between the bulls. The 7% glycerol concentration showed the best result regarding post dilution motility, post thaw motility, progressive motility and velocity.

Conflict of Interest

The authors have not declared any conflict of interest.

Authors Contributions

All the authors contributed equally in the manuscript.

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