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INTERACTIONS OF DILUENTS, CRYOPROTECTIVE AGENTS AND STRAW FILLING CAPACITY ON QUALITY AND FERTILIZING ABILITY OF BUFFALO SEMEN

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SUMMARY

The present study was carried out on three buffalo bulls. Their semen was extended in three diluents (Milk, Laiciphos and Tris) with three cryoprotective agents (Glycerol, DMSO and propylene glycol, PG) packaged in either mini (0.25ml) or medi (0.50 ml) straws. Significant differences in the percentages of post-thawing motility (PTM), intact acrosome (PIA) and hypoosmotic swelling test (HOST) were observed for semen extended in Milk diluent (52.80±0.39%; 95.40±0.43% and 17.97±0.27%, respectively), Laciphos (48.33± 0.48%; 94.50 ±0.29% and 15.77±0.22%, respectively) and Tris (46.23 ± 0.49%; 92.13±0.37% and 14.43 (0.21% respectively). The semen quality test (SQT) appeared significantly higher for milk diluent (2.13±0.11) than Tris and Laiciphos diluents (1.77±0.05 and 1.72± 0.06, respectively). The capacity of straws had a significant effect on PIA and SQT of mini straws (94.78±0.33 and 16.29±0.30, respectively) compared to medi straws (93.20±0.35% and 15.87± 0.28 respectively). Significant effects were recorded for cryoprotective agents on PTM, PIA and HOST in semen cryoprotected with glycerol (51.14± 0.52%; 95.33 (0.3% and 17.00±0.38 respectively) compared to DMSO (49.03± 0.56; 94.27 ± 0.44% and 16.07± 0.29 %, respectively) and PG (47.20±0.75%; 92.47±0.93% and 15.10±0.38%, respectively). The conception rates (CR) showed non significant differences among semen extended in Milk diluent (60.78%), Laiciphos (48.00%) and Tris (43.40%) and among glycerol (53.70%); DMSO (50.98%) and PG (46.94%) as cryoprotective agents and between mini (52.50%) and medi (48.65%) straws. Meanwhile a higher CR (57.14%) was recorded for semen extended in Milk diluent cryoprotected with glycerol and packaged in mini straws. From the present study

it can be concluded that, the use of milk extender for freezing buffalo semen packaged in mini straws is an efficient method for obtaining good quality semen and high fertility rate.

INTRODUCTION

With the worldwide extension of the frozen semen; several investigations had been done to maximize the rate of conception by modifying processes of the freezing technique. One of these processes, is the availability of many efficient extenders (Rasul et al 2000 and Pavlenz et al 2002). Not only the available extender, but also the most cheapest and save cryoprotective agents should be considered (Kumar et al 1992 and Fabbrocini et al 2000) in addition to the straw-filling capacity (Kreindlin 1985, Amann and Pickett 1987 and Graham 1999). However, in spite of establishing both the favourable extender and cryoprotective agents, the conception rate of cows and buffaloes under the local condition is still unsatisfactory (El-Naggar 1999 and El-Hakem, 2002). The fertility could be predicted through some laboratory tests including post-thaw motility, percentage of intact acrosome, the hypoosmotic swelling test and semen quality test (Correa and Zavos 1994;Reddy and Bordekar 1999 and Meyer and Barth 2001). The present work aimed to study the effect of some available extenders associated with more efficient cryoprotective agent in freezing buffalo semen and their consequence on its quality and fertilizing capacity.

MATERIALS AND METHODS

1-Animals:

The present study was carried on three sexually mature buffalo bulls kept on the experimental farm of faculty of agriculture Moshtohor, Kaliboia, during the period from August 2002 to May 2003. All animals were free from any apparent diseases and external parasites. They were raised under convential condition of nutrition and management.

2- Chemicals and reagents:

2.1. Diluents (Skim milk; Laiciphos 488, imv, Technologies, France and Tris) and 20% egg yolk and antibiotics were added to each diluent before dilution (Foote, 1972,Abdel-Malak et al.1993 and Ziada et al. 1995).

2.2. Cryoprotective agents as Glycerol 7%; Dimethyl-sulphoxide, DMSO 5% and propylene glycol, PG 6% (Meryman et al.1971).

2.3. Spermac stain (Sertipro N.V.,Belgium) for detection of intact acrosomes(Oettle,1986).

2.4. Hoypoosmotic solution, used for HOST(Jeyendran et al., 1992).

2.5. Semen quality test, SQT (Biodiagnostic Comp , Cairo ,Egypt) for studying the metabolic activity of spermatozoa(Reddy and Bordekar ,1999).

3-Semen collection and initial evaluation:

Semen was collected once a week by means of thermo-regulated (42°C) artificial vagina (A.V.). The collected semen was transferred to the laboratory of A.I. within few minutes and kept in water both at 35oC for evaluation. Aliquots of each semen sample were taken and immediately evaluated against forward progressive motility and sperm cell concentration.

4- Semen processing:

Only ejaculates of at least 600 x 10^6 sperm / ml concentration and more than 70% initial motility was used. Semen samples were extended in three buffers (skimed milk, Laiciphos, 488 and Tris-fructose) with three cryoprotective agents (Glycerol 7%, DMSO 5% and PG 6%).

The rate of dilution was 1: 10 for semen packaged in medi straws and 1 : 4 for those packaged in mini straws. On processing, semen was extended in two portions for each diluent, cooled to 5°C within one hour and equilibrated at 5°C for another one hour. Straws were filled and sealed manually. After equilibration, the extended semen was frozen on liquid nitrogen vapor for 10 minutes inside a foam box (50x 34x 25 cm, containing 8 liter of liquid nitrogen) at a height of 5 cm above the level of liquid nitrogen and provided with heater for activation of the liquid nitrogen. The straws were then immersed in liquid nitrogen for storage. After 24 hours, the frozen straws were thawed at 35°C for 0.5 minutes.

5- Semen assays:

The thawed semen was examined for percentage of post-thawing motility (PTM), percentage of intact acrosome(PIA) ,Hypo-osmotic swelling test (HOST) and Semen quality test (SQT).

Semen quality test depends on the ability of metabolic activity of spermatozoa to reduce the resazurin dye (blue) with maximum absorption at 615 nm, to resorufin with pink colour with maximum absorption at 580nm (Resazurin Reduction test, RRT). Absorption spectra were scanned for resazurin and resorufin. The ratio of the two optical densities was used as a probe to discriminate the various grades of semen samples. In azoospermic samples, RRT ratio ranges from 0.7 to 1.16, in oligospermic samples from 1.5 to 2.0 and in normospermic and proven fertile samples from 2.25 to 5.9 (Dart et al., 1994 , Foote 1999 and Reddy and Bordekar 1999).

6- Field fertility:

Attempts of insemination of 154 buffaloes (80 were inseminated with mini straws and 74 with medi straws) in three villages in Kaliobia province were undertaken. Pregnancy was diagnosed by rectal palpation 60 days after A.I.

7-Statistical analysis:

The obtained data were tabulated and subjected to analysis of variance (ANOVA), student t test and chi-square according to computer program (SAS, 1987).

RESULTS

In an overall mean (Table, 1), the percentage of post-thawing motility (PTM) of buffalo bull spermatozoa packaged in mini straws (49.80± 0.53%) did not significantly higher than that of medi straws(48.44 ± 0.56%). Irrespective of the effect of straw filling capacity, PTM of buffalo bull spermatozoa varied significantly ($P < 0.01$) between semen extended in Milk (52.80 ± 0.39%), Laiciphos (48.33 ± 0.48 %) and Tris (46.23 ± 0.49%) extenders. Regardless of the extender, significant differences were observed in the PTM on using glycerol (51.14 ± 0.52 %) compared to DMSO (49.03 ± 0.56 %) and propylene glycol (47.20 ± 0.75 %)as cryoprotective agents.

Table (1): Effect of diluents, cryoprotective agents and straw filling capacity on the percentage of post-thawing motility (PTM) of frozen buffalo spermatozoa (M±SE).

Factor	Mini Straws	Medi Straws	Overall mean
Milk			
Glycerol	55.00 ± 0.55	53.40 ± 0.93	54.20 ± 0.57
DMSO	53.20 ± 0.70	51.00 ± 0.84	52.10 ± 0.71
PG	52.40 ± 0.52	51.80 ± 0.80	52.10 ± 0.57
Mean	53.53 ± 0.52	52.07 ± 0.53	52.80 ± 0.39(a)
Laiciphos			
Glycerol	50.80 ± 0.37	50.00 ± 1.05	50.40 ± 0.54
DMSO	49.20 ± 0.58	48.40 ± 0.51	48.80 ± 0.39
PG	47.20 ± 1.02	44.40 ± 0.68	45.80 ± 0.72
Mean	49.07 ± 0.55	47.60 ± 0.75	48.33±0.48(b)
Tris			
Glycerol	49.00 ± 0.45	48.60 ± 0.93	48.80 ± 0.49
DMSO	46.80 ± 0.97	45.60 ± 0.81	46.20 ± 0.63
PG	44.60 ± 0.51	42.80 ± 0.73	43.70 ± 0.52
Mean	46.80 ± 0.60	45.67 ± 0.77	46.23 ± 0.49 (c)
Overall			
Glycerol	51.60 ± 0.80	50.67 ± 0.67	51.14 ± 0.52(A)
DMSO	49.73 ± 0.84	48.33 ± 0.71	49.03 ± 0.56 (B)
PG	48.07 ± 0.97	46.33 ± 1.12	47.20 ± 0.75 (BC)
Mean	49.80 ± 0.53(a)	48.44 ± 0.56(a)	49.12 ± 0.39

- Each experiment was repeated 5 times.
- Overall means with different superscripts within the same column/raw for the same factor differed significantly at least at $p< 0.05$.

As shown in table 2, there were significant differences in the percentage of intact acrosome (PIA) between mini straws (94.78 ± 0.33 %) and medi straws (93.29 ± 0.35%) and between glycerol (95.33 ± 0.31%) and DMSO (94.27 ± 0.44%) in comparison to propylene glycol (92.47 ± 0.39%) as cryoprotective agents. Moreover significant differences ($p<0.01$) in the PIA were observed for spermatozoa extended in Milk (95.40 ± 0.43%) compared to that extended in Laiciphos (94.50 ± 0.29%) and Tris (92.13 ± 0.37%).

Table (2): Effect of diluents, cryoprotective agents and straw filling capacity on the percentage of intact acrosome (PIA) of frozen buffalo spermatozoa (M±SE).

Factor	Mini Straws	Medi Straws	Overall mean
Milk			
Glycerol	97.80 ± 0.20	95.40 ± 0.20	96.60 ± 0.64
DMSO	97.60 ± 0.24	95.20 ± 081	96.40 ± 0.54
PG	93.60 ± 0.51	92.80 ± 0.86	93.20 ± 0.49
Mean	96.33 ± 0.55	94.47 ± 0.57	95.40 ± 0.43(a)
Laiciphos			
Glycerol	96.00 ± 0.51	95.00 ± 0.32	95.50 ± 0.64
DMSO	95.40 ± 0.55	93.00 ± 0.84	94.20 ± 0.52
PG	94.40 ± 0.24	93.200 ± 0.58	93.80 ± 0.47
Mean	95.27 ± 0.30	93.73 ± 0.41	94.50 ± 0.29 (B)
Tris			
Glycerol	94.00 ± 0.32	93.80 ± 0.37	93.90 ± 0.26
DMSO	92.80 ± 0.37	91.40 ± 0.75	92.10 ± 0.46
PG	91.40 ± 0.75	89.40 ± 0.60	90.40 ± 0.56
Mean	92.80 ± 0.42	9153 ± 0.58	92.13 ± 0.37 (c)
Overall			
Glycerol	95.00 ± 0.46	94.73 ± 0.37	95.33 ± 0.31 (A)
DMSO	95.27 ± 0.56	93.33 ± 0.58	94.27 ± 0.44 (A)
PG	93.13 ± 0.45	91.80 ± 0.59	92.47 ± 0.39 (B)
Mean	94.78 ± 0.33(a)	93.29 ± 0.35(b)	94.02 ± 0.25

- Each experiment was repeated 5 times.
- Overall means with different superscripts within the same column / raw for the same factor differed significantly at least at $p< 0.05$.

In an overall mean (Table 3), the HOST of buffalo bull spermatozoa packaged in mini straws (16.29 ± 0.30%) did not significantly higher than those packaged in medium straws (15.82 ± 0.28%). Generally, the HOST of buffalo bull spermatozoa differed significantly ($p < 0.05$) between semen extended in Milk (17.97 ± 0.27%) when compared to those extended in Laiciphos (15.77± 0.22%) and Tris (14.43 ± 0.21%) extenders. Regardless of the extender, a non-significant difference was observed in the HOST on using either glycerol (17.00 ± 0.38%) or DMSO (16.07 ± 0.29%) as cryoprotective agent. Meanwhile, HOST appeared significantly lowered on using propylene glycol (15.10 ± 0.38%) when compared to those of glycerol and DMSO.

Table (3): Effect of diluents, cryoprotective agents and straw filling capacity on the HOST of frozen buffalo spermatozoa (M±SE).

Factor	Mini Straws	Medi Straws	Overall mean
Milk			
Glycerol	19.80 ± 0.49	19.00 ± 0.45	19.40 ± 0.34
DMSO	18.20 ± 0.37	17.00 ± 0.45	17.60 ± 0.34
PG	17.20 ± 0.37	16.60 ± 0.51	16.90 ± 0.31
Mean	18.40 ± 0.36	17.53 ± 0.38	17.97 ± 0.27
Laiciphos			
Glycerol	16.60 ± 0.60	16.40 ± 0.60	16.50 ± 0.40
DMSO	16.00 ± 0.45	15.60 ± 0.60	15.80 ± 0.36
PG	15.00 ± 0.32	15.00 ± 0.465	15.00 ± 0.26
Mean	15.87 ± 0.31	15.67 ± 0.33	15.77 ± 0.22(b)
Tris			
Glycerol	15.20 ± 0.37	15.00 ± 0.32	15.1± 0.23
DMSO	14.80 ± 0.58	14.80 ± 0.37	14.80 ± 0.33
PG	13.80 ± 0.37	13.00 ± 0.45	13.40 ± 0.31
Mean	14.60 ± 0.29	14.27 ± 0.32	14.34± 0.21(c)
Overall			
Glycerol	17.20 ± 0.58	16.80 ± 0.51	17.00 ± 0.38(A)
DMSO	16.33 ± 0.45	15.80± 0.35	16.07 ± 0.29(A)
PG	15.33 ± 0.42	14.87 ± 0.47	1510 ± ± 0.38(B)
Mean	16.29 ± 0.30(a)	15.87 ± 0.28(a)	16.06± 0.2

- Each experiment was repeated 5 times.
- Overall means with different superscripts within the same column / raw for the same factor differed significantly at least at $p < 0.05$.

From table 4, semen quality test (SQT) of buffalo bull spermatozoa significantly differed ($P < 0.01$) for semen packaged in mini straws (2.24±0.06) compared to that of medi straws (1.54 ± 0.03). Regardless the effect of straw size, SQT of buffalo bull spermatozoa increased significantly for semen extended in Milk (2.13 ± 0.11) when compared to those extended in both Laiciphos (1.77 ± 0.06) and Tris (1.77 ± 0.05) extenders. Non-significant differences in SQT were recorded on using glycerol, DMSO and propylene glycol as cryoprotective agents.

Table (4): Effect of diluents, cryoprotective agents and straw filling capacity on the SQT of the frozen buffalo spermatozoa (M±SE).

Factor	Mini Straws	Medi Straws	Overall mean
Milk			
Glycerol	2.78 ± 0.03	1.63 ± 0.03	2.20 ± 0.19
DMSO	2.71 ± 0.04	1.60 ± 0.11	2.16 ± 0.19
PG	2.61 ± 0.03	1.47 ± 0.05	2.04 ± 0.19
Mean	2.70 ± 0.02	1.57 ± 0.04	2.13 ± 0.11(a)
Laiciphos			
Glycerol	2.18 ± 0.10	1.66 ± 0.09	1.92 ± 0.11
DMSO	2.05 ± 0.06	1.50 ± 0.03	1.77 ± 0.10
PG	1.83 ± 0.05	1.41 ± 0.07	1.62 ± 0.08
Mean	2.02 ± 0.06	1.52 ± 0.04	1.72 ± 0.06(b)
Tris			
Glycerol	2.01 ± 0.08	1.62 ± 0.09	1.98 ± 0.09
DMSO	1.99 ± 0.13	1.58 ± 0.10	1.79 ± 0.10
PG	1.97 ± 0.09	1.43 ± 005	1.70 ± 0.10
Mean	1.99 ± 0.05	1.45 ± 0.05	1.77 ± 0.05(b)
Overall			
Glycerol	2.32 ± 0.10	1.64 ± 0.04	1.98 ± 0.08(A)
DMSO	2.25 ± 0.10	1.56 ± 0.05	1.91 ± 0.08(A)
PG	2.14 ± 0.10	1.44 ± 0.03	1.79 ± 0.08 (A)
Mean	2.24 ± 0.06(a)	1.54 ± 0.03 (b)	1.84 ± 0.05

- Each experiment was repeated 5 times.
- Overall means with different superscripts within the same column / raw for the same factor differed significantly at least at $p< 0.05$.

There were non-signifiant differences in conception rate between mini and medi straws, Milk, Laiciphos and Tris diluents and glycerol, DMSO and PG as cryoprotective agents (table 5).

Higher conception rate was recorded for semen packaged in mini straws (52.50%) than that packaged in medi straws(48.65%). Moreover, semen extended in Milk diluent had higher conception rate (60.78%) in comparison to those extended in Laiciplos (48.00%) and Tris (43.40%). Additionally, the conception rate of frozen semen cryoprotected with glycerol showed higher percentage (53.70%) than that cryoprotected with DMSO (50.98%) and PG (46.94%).

Table (5): Effect of diluents, cryoprotective agents and straw filling capacity on the conception rate for frozen buffalo spermatozoa.

Factor	Mini Straws		Medi Straws		Overall mean	
	NO.	CR %	NO.	CR %	NO.	CR %
Milk						
Glycerol	6/9	66.66	5/8	62.50	11/17	64.71
DMSO	5/8	62.56	5/8	62.50	10/16	62.50
PG	5/9	55.45	5/9	55.56	10/8	55.56
Mean	16/26	61.45	15/25	42.86	31/51	60.78
Laiciphos						
Glycerol	5/9	55.00	4/8	50.00	9/17	52.94
DMSO	5/10	50.00	3/7	42.86	8/17	47.06
PG	4/9	44.44	3/7	42.86	7/16	43.75
Mean	14/28	50.00	10/22	45.45	24/50	48.00
Tris						
Glycerol	5/10	50.00	4/10	40.00	9/20	45.00
DMSO	4/9	44.44	4/9	44.44	8/18	44.44
PG	3/7	42.86	3/8	37.50	6/15	40.00
Mean	12/26	46.15	11/27	40.74	23/53	43.40
Overall						
Glycerol	16/28	57.14	13/26	50.00	29/54	53.70
DMSO	14/27	51.85	12/24	50.00	26/51	50.98
PG	12/25	48.00	11/24	45.83	23/49	46.94
Mean	42/80	52.50	36/74	48.65	78/154	50.65

-Non significant differences were recorded after chi square test

DISCUSSION

The present study showed a significant increase in PTM, PIA and HOST of buffalo semen extended in Milk and Laiciphos than that in Tris diluent. These findings came in accordance with Kumar et al (1992) and Galli et al (1993) who found that, comparison of diluents based on PTM of spermatozoa, milk was better than Tris diluent. These differences could be attributed to that, milk contains an abundant supply of casein which is antioxidant and improve sperm motility immediately after freezing and thawing as well as fertility (Foote et al., 2002). On the other hand, Ziada et al (1995) found that, post thawing motility of buffalo bull spermatozoa did not vary significantly in Laiciphos, Skim milk and Tris-citric acid fructose diluents. Moreover, Watson (1995); Rasul et al (2000) and Pavlenze et al (2002) reported that post thawing quality of bull, buffalo bull and ram semen can be improved by using Tris based buffering system. Such differences could be attributed to individual variations, processing technique and post thawing treatment. (Berndtson et al 1981 and El-Hakem 2002).

The present study revealed a significant increase ($P < 0.01$) in PIA of buffalo spermatozoa extended in Milk and Laiciphos compared to those extended in Tris. Kakar and Anand (1984) observed a great acrosomal damage occurred in buffalo spermatozoa after freezing and thawing. Regardless of type of diluent, processes of freezing cause acrosomal damage to buffalo spermatozoa (Chinnaiya and Ganguli, 1980 and El-Sheltawi et al 1995), shrinkage of sperm plasma mumbrane (Allam 2003), and increased the incidence of bent tails (El-Sheltawi et al 1993). Additionally, fluidization of sperm cell plasma membrane causes release of intracellular constituents (Sarmah et al 1984).

The differences in PTM and PIA might be due changes in osmolarity of different diluents, in addition to less resistance of buffalo spermatozoa to prefreezing procedure than cattle spermatozoa (Verma et al 1975). It had been believed that, buffalo spermatozoa inherently more fragile and sensitive to freezing (El-Azab et al 1998b and Sanson et al 2000), had more extensive damage of acrosome and subsequent loss of motility (Galli et al 1993). El-Azab et al (1998b) and Rasul et al., (2000) reported that poor freezability may be correlated to the low membrane phospholipid content and its loss during freeze-thawing or inmunogenetic factor .

The hypoosmotic swelling test (HOST) appeared significantly differed ($p<0.01$) for semen extended in different diluents (Milk, Laiciphos and Tris) . The abrupt changes in osmotic pressure result in occurrence of osmotic shock, reduced sperm viability and sperm membrane damage (Correa et al., 1997). The age of bulls, processing technique and

handling of frozen semen may be incrminated (Youssef 1997) or due to immunological factors (El-Azab et al., 1998b). Integrity of acrosomal cap has been positively correlated with fertility in bovine (Saacke and White 1972). It has been reported that, the first changes due to the processes of freezing are distension and loosening of the peri-acrosomal plasmalemma of the spermatozoa followed at late steps by ruffling and swelling plasmalemma overlying the post-acrosmal sheath; abrupt changes in osmotic pressure results in osmotic shock, reduced sperm viability and sperm membrane damage (Correa et al., 1997). The current study showed significant increase in HOST reaction for semen extended in Milk compared to those in Laiciphos and Tris diluents. On the other hand Pavlenz et al., (2002) found that Tris- based extenders preserved better ram sperm viability, acrosome, membrane integrity and consequently capacitation. In the same aspect, Correa and Zavos (1994) and Abdel-Ghaffar et al (1999) indicated that, ratio of acrosin activity to percentage of intacted acrosome and hypoosmotic-swelling test could be used as an additional tool for evaluation of fertilizing ability and predicting the fertility. While Rasul et al., (2000) reported that, plasma membrane integrity and normal acrosome of buffalo spermatozoa did not differ due to buffering system. Moreover Rota et al .,(2000) reported that, HOST does not appear to be sufficiently sensitive to discriminate between semen samples of intermediate fertility in bulls.

The SQT appeared significantly higher for buffalo spermatozoa extended in Milk diluent and non-significant variations were recorded between Laiciphos and Tris. The SQT is highly correlated with sperm concentration and the percentage of motile sperm from several species (Dart et al 1994 and Mahmoud et al 1994). Moreover it reflects the metabolic activity of the sperm cell such as ATP and measures the undesirable components or contaminants of sperm such as reactive oxygen species and neutrophils (Zalata et al 1995 and Foote 1999).

The capacity of straws had significant differences on PIA and SQT between mini and medi straws. The present difference could be due to that, the post thaw sperm quality improves as package volume decrease (Cassou 1968, and Kreindlin,1985). Freezing in small volumes permits more sperm to be cooled at the optimal cooling rate, than freezing in large volumes, where the cooling rate for the sperm at the surface of the sample will be faster than for sperm located in the center of the sample (Amann and Pickett 1987 and Graham 1999). Regarding the effect of cryoprotective agents, there were significant differences in the PTM, PIA and HOST reaction between glycerol, DMSO and PG. It has been emphasized that glycerol is the least deleterious cryoprotective agent (Wall and Foote 1999), followed by dimethyl acetomide and dimethyl sulfoxide in bovine (Snedeker and Gaunya 1970) and rabbit (Hanada and Nagase,

1980). In buffaloes, Fabbrocini et al., (2000), reported that, the composition of the extender in which semen is diluted before freezing play a major role in successful cryopreservation of spermatozoa. Substances of high osmolarity with low molecular weight and high solubility like glycerol protect sperm cells during freezing process. In the same respect Hammerstedt et al (1990) and Holt (2000) reported that, glycerol would alter the bioenergetic status of spermatozoa, perhaps interfering with the balance between ATP synthesis and utilization.

Higher conception rate was recorded for semen packaged in mini straw (52.50%) than that packaged in medi straw (48.65%). Moreover, semen extended in Milk diluent had higher conception rate (60.78%) in comparison to those extended in Laiciphos (48.00%) and Tris (43.40%). Additionally, the conception rate of frozen semen cryoprotected with glycerol showed higher percentage (53.70%) than that cryoprotected with DMSO (50.98%) and PG (46.94%). These differences might be attributed to the higher post thawing motility and intacted acrosome (Youssef 1997 and El-Hakem 2002). The higher non-return rate may be due to the presence of increased amounts of energy rich materials in milk diluents as compared to other diluents (Salisbury et al.1978 and Allam 2003). On the other hand. Rasul et al (2000), Pavlenz et al (2002) and Thun et al (2002) recorded higher CR for frozen semen extended in Tris.

Inscoping, the effect of cryoprotective agent, on CR the present study showed higher CR with glycerol in comparison to DMSO and PG. These findings came in agreement with Fabbrocini et al., (2000). The higher CR for semen extended with glycerol attributed to the least deleterious effect of glycerol in comparison to DMSO Snedeker and Gaunya (1970) and PG (El Azab et al 1998a). In the same aspect, Revell (1994) found a correlation of r = + 0.79 between HOST results and fertility (expressed as non-return to estrus rates) when the bull effect was removed. These results might be attributed to species differences, individuality, processing technique, storage and handling of frozen semen (Abdou 1987 and El Azab et al., 1998a& b). From the present study it could be concluded that processing of buffalo semen using milk egg yolk extender with glycerol and packaged in mini straws gave the best semen quality and field fertility..

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