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Comparison the Effects of CUE, IVT, Minnesota and TRIS Extenders on Viability of Spermatozoa in Frozen Semen Obtained from Two and Seven Years Old Male Buffaloes

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Abstract: Upon the results of this study, mean motility of spermatozoa before and after dilution in different extenders and also sperm concentration were very significantly differ ($p<0.01$). Mean percentage of motile sperm of 7 old buffaloes pre-freeze and post-thaw in Cornell and TRIS extenders comparing with IVT and Minnesota extenders in 2 years old buffaloes have very significant difference($p<0.01$). The mean percentage of abnormal and dead spermatozoa with IVT and Minnesota extenders in 2 years old buffaloes comparing with Cornell and TRIS extenders in 7 old buffaloes have very significant difference($p<0.01$). Interactions between ages of buffaloes and extenders type in different parameters were very significant ($p<0.01$). But interactions between extenders type and weeks of experiment and also between ages of buffaloes and weeks of experiment and triple interactions were not significant ($p>0.05$).

Key words: Buffalo, extender, sperm, TRIS, IVE, CUE, minnesota

INTRODUCTION

Today, there are about 151.5 million water buffalo in the world. Of these about 96.6 % are found in Asia. For a thousand years or more, this important animal species has provided draft power, milk, meat and hide to millions of people, particularly small-scale farmers (Johar *et al*., 1976).

Today, according to increasingly growth of world population, supplying required animal protein (milk and meat) has become very important. But with regard to low genetically potential of many of animals in undeveloped and developing countries, produced protein materials against the costs is very low and rearing the low productive animals is not economical. Today animal breeding, using new methods, could increase widely the mean of production of low producing native animals by crossing them with exotic improved bulls. One of these used methods for widely application of the sperm of exotic excellent breeds is the using of Artificial Insemination (AI) technique and modern semen dilution methods. Using this technique, per ejaculation of an excellent bull can used to inseminate 300-400 cows. Therefore, possibility of using per ejaculate of an excellent bull widely in herd becomes possible.

Extenders for treating ejaculated semen are very important, so success in this area depends on cryopreservation ability of extender. Since past TRIS, (Austin *et al*., 1992; Jainudeen and Santhana, 1982; Mohan and Sahi, 1991) IVT (Azawi *et al*., 1993; Hassan and Graham, 1994; Johar *et al*., 1974; Suzuki and Niwa, 1981). CUE (Austin *et al*., 1992; Sahni and Roy, 1972) and Minnesota extenders (Sexton and Giesen, 1982, 1983) have used in USA for animals such as cattle, pig and fowls, but using of these types of extenders for diluting in freezing condition for buffalo semen, have not been used.

In all the earlier studies, only post thawing sperm motility was used as the criterion for evaluation. A comprehensive study using different extenders was lacking. Hence, an attempt has been made to study the relative efficacy of four extender for freezing of buffalo semen based on sperm motility and acrosomal integrity.

MATERIALS AND METHODS

In this study, 4 head two years old male buffaloes with mean weight 307±8.5 kg and 4 head seven years old male buffaloes with mean weight 710 + 10.9 kg were used. According to the effects of management and feeding on quantity and quality of ejaculated semen, before experiment period, animals separated and reared under unique nutrition and management program. After a month for adaptation, ejaculates were collected weekly with an artificial vagina for a month. Sixteen ejaculates from four mature buffalo bulls of Murrah breedwere test in a 4×2×4 factorial combination. Different extenders' formulas presented in Table 1-3.

After preparation, each of extenders splinted to two equal parts and then 7 volume percentage glycerol was

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Table 1: Composition of CUE and IVT extenders

Ingredients	IVT	CUE
Sodium bicarbonate (g)	0.21	0.21
Sodium citrate (g)	2	1.45
Potassium colurid (g)	0.04	0.04
Glucose (g)	0.3	0.3
Sulfanilamide (g)	0.3	0.3
Penicillin (IU mL^{-1})	1000	1000
Streptomycin ($\mu g\ mL^{-1}$)	1000	1000
Glycin (g)	1.92	1.92
Egg yolk (g)	10	20
Buffer (g)	90	80

Table 2: Composition of Minnesota extender

Ingredients	Minnesota
Adnitol (g)	1
Sorbitol (g)	1
Manitol (g)	10
Eritrol (g)	10
Delsitol (g)	10
Dextrose (g)	10
Fructose (g)	3
Inozitol (g)	10
Sodium citrate (g)	2.7
Citric acid (g)	0.4

Table 3: Composition of TRIS extender

Ingredients	TRIS
Hydroxyl methyl amino methane (g)	3.028
Fructose (g)	1
Water (g)	75
Citric acid (g)	0.1

added to one of them. Pre-freeze Sperm motility was determined by Differential Coloration method.

All chemical used in the preparation of extender were purchased from Sigma (St. Louis, MO, USA). Semen from buffalo bulls, maintained at the Buffalo Research, Station Research center, URMIA, IRAN was collected at weekly interval with the help of artificial vagina 42° C during the months of November and December 2001. Two consecutive ejaculates were obtain from each bull per day of experiment. Semen ejaculates with more than 60% sperm motility, regardless of bull and ejaculates (first, second or both), were selected for further processing. The qualified ejaculates, of all four bulls, were pooled to obtain sufficient semen volume for one replicate and to eliminate bull effect. The pooled semen was kept at 37°C for 15 minute (holding time). This study was repeated four time. Each pooled semen sample was divided into 4 aliquots that, they were extended (1:10) at 37°C with TRIS, Cornell, Illinoise and Minnesota extenders and cooled to 4 °C in 2.5h. After equilibration semen was packaged in 0.5 mL Franch straws and frozen in a programmable cell freezer (KRYO 10 Series 111, Planer, Sunbury-on-Thames, Middlese, UK) using the following freezing rate +4° to -15°C at the rate of -3°C/min and then from -15°C to -80°C at the rate of -10°C/min. After reaching -80 °C the frozen straws were plunged into liquid nitrogen and stored for at least 24h before evaluation. Semen straws were thawed at 37°C for 50 sec and post thaw semen quality was evaluated by the following semen assays.

An aliquot of semen (5μ) was placed on a prewarmed (37°C) Makler chamber (depth 10 μm; Sefi-Medical industries, Haifa, Israel) and analyzed for sperm motion characteristics using a computer- assisted sperm analyzer (CASA; SM-CMA; Mika Medical, Germany). Each semen sample was assessed for overall motility (S-Mot, %); linear motility (LIN-Mot, %; the motile sperm moving in straight-liner). The validation of CASA for buffalo sperm has already been reported by Rasul *et al.* (2000).

Manual/visual sperm analyses: Initially, semen assay were conducted by placing a sub-sample from the semen sample on a microscope slide and visually evaluating the sub- sample. These types of assays using fresh or fixed, unstained or stained sub-samples, remain a mainstay of the assays conducted by most laboratories.

Unstained spermatozoa: Fresh unstained spermatozoa are commonly examined microscopically, to estimate the percentage of motile sperm in semen sample. Such estimations can include both the percentages of motile cells, as well as progressively motile cells. In addition, sub-samples can be fixed with formaldehyde or gluteraldehyde and the morphology of the cells evaluated using phase contrast or Differential Interference Contrast (DIC) microscopy (Graham, 1996).

Stained spermatozoa: These analyses usually require a spermatozoa sub-sample to be stained to enhance visualizing the sperm (IVF or zona binding assays) or differences in organelle structure (dried spermatozal smears on a microscope slide) that can be evaluated visually using microscopy (Cross and Meizel, 1989).

RESULTS AND DISCUSSION

Upon the results achieved by this study, the average motility of the sperm before and after dilution, in several extenders, in buffalos with different ages (2 and 7 years old) had a very significant variance ($p<0.01$).

The comparison of the mean percentage of the sperm motility of the sperm, in several dilutors (pre-freezing) in 2 and 7 years old buffalos, shows that the percentage of sperm motility after dilution and per-freezing in 7 years old buffalos is significantly more than 2 years old ones ($p<0.01$). On the other hand upon the Duncans test, mean percentage of sperm's motility in

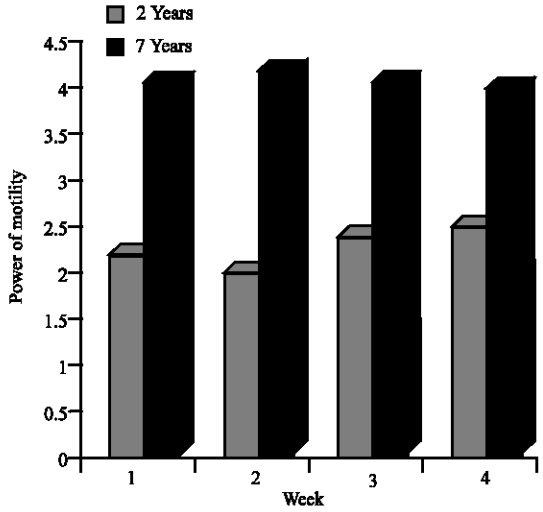

Fig. 1: Comparison between motility of sperm, before dilution in 2 and 7 years old buffalos, in several weeks

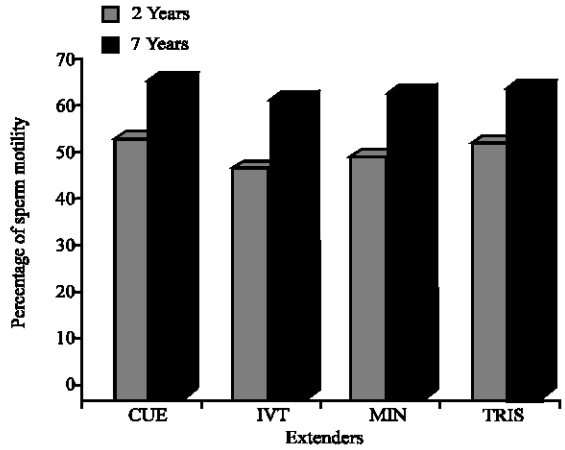

Fig. 2: Percentage of sperm motility in 2 and 7 years old buffalos, before freezing

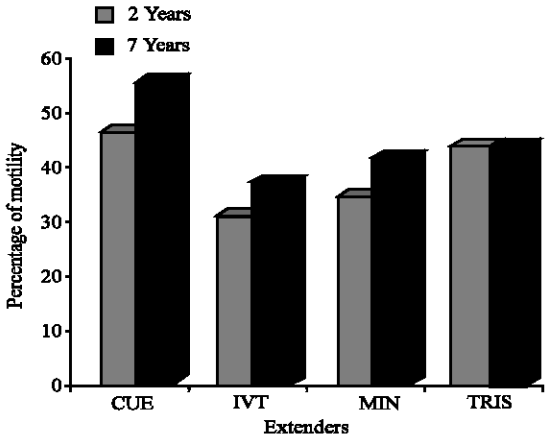

Fig. 3: The mean motility percentage of sperms after freezing, in several kinds of dilutors in 2 and 7 years old buffalos

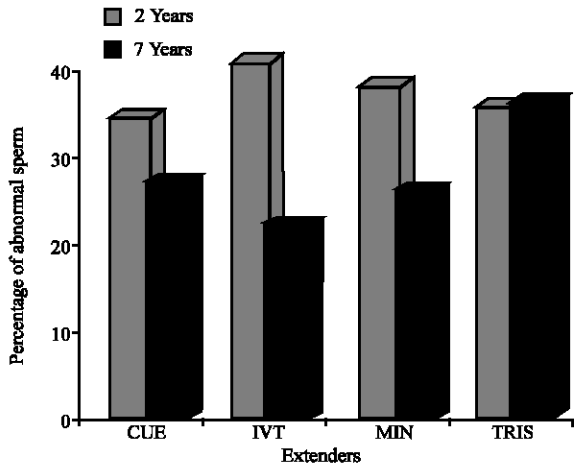

Fig. 4: Percentage of dead and abnormal sperms among two groups of buffalos (2 and 7 years old)

Cornell dilutor (CUE) in comparison with the other extenders, among both groups of buffalos, were more (Fig. 1 and 2).

Figure 3 shows the mean motility percentage of sperm among the 2 and 7 years old buffalos, after freezing. From view point of motility percentage, there has been a significant difference between 2 and 7 years old buffalos ($p<0.01$). Duncan tests to compare, mean percentage of motility after freezing showed that this factor in Cornell extender have had a more significance among both groups of buffalos (2 and 7years old).

The mean percentage of dead and abnormal sperms, in 2 and 7 years old buffaloes is indicated separately, in Fig.4. As it is realized in this figure average percentage of dead and abnormal sperms in 2 years old buffalos, is significantly more than that of 7 years old buffalos ($p<0.01$). Also, dead sperms in both groups (2 and 7 years old) in IVT extender is more than others. Comparison of mean percentage of dead sperms in 2 years old buffalos, using Duncan tests, shows that preservation ability of IVT extender is more than the other kinds, even MIN extender.

Figure 5 shows the percentage of alive and forward moving sperms, in several kinds of extenders ' for each age (2 and 7 years old) separately_during the experiment. There is a very significant difference in mean percentage of alive and forward moving sperms, between all of extenders during four weeks ($p<0.01$) as either percentage of mean of alive and forward moving sperms is very

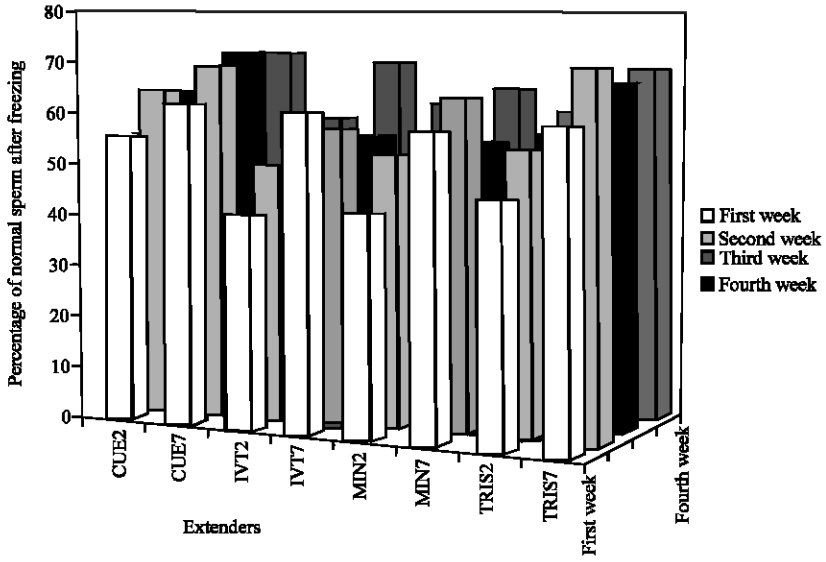

Fig. 5: Comparison of effect of several dilutors on mean percentage of sperm alive after freezing for each age

significantly different ($p<0.01$) and also percentage of alive and active sperms in CUE extender was more. The comparison of mean percentage of alive and active sperms in CUE extender had a very significant difference ($p<0.01$).

According to Fig. 3, the results of this study conform to results achieved by Austin *et al.* (1992). In a general point of view to the ingredients of IVT and CUE dilutors it seems that, there is no much difference in the ingredients, except Sodium citrate Yolk mass, clicine.

According to the reports of Bearden and Fuquay (1990), decrease in metabolic activity of sperms in plasma of semen is related to high concentration of CO_2.

Because there was not required equipments to inject CO_2 in the location of experiment, probably these changes are originated from lack of CO_2 in IVT ingredients and this is conformed to the reports which were presented by Pursel *et al.* (1974). The researches done by Sexton (1988), about Minnesota dilutor showed that sperm of turkey diluted by Minnesota dilutor in comparison with the other kinds of dilutor such as BPSE1 and IMV2 had an undesirable quality. Mount of sperms motility, 24 h after storage in Minnesota dilutor was around 38% and the damage related to the same dilutor was around 23% (Johar *et al.*, 1976). And more over, the sugars used in the structure of this dilutor are so expensive, that in a commercial point of view, produce and usage of this dilutor is not economical. The results show that, firstly quality and quantity of sperms produced by 7 years old buffalos in comparison with 2 years old buffaloes is significantly better ($p<0.01$). From the other point of view, in comparison with the other kinds of dilutor, maintenance ability of CUE dilutor was better ($p<0.01$).

SUGGESTIONS

According to the results, it is recommended to that the quantitative and qualitative characteristics of sperms in 3-6years old buffaloes must be studied.

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REFERENCES

Austin, A., L.P. Thangaraja and Y. Ulagantahan, 1992. Effect of TRIS Hydroxy Methyl Aminomethane Sodium citrate, Cornell University Extender and Lactose on cytomorphology of bull spermatozoa during storage. Indian Vet. J., 69: 408-415.

Azawi, O.I., A.L. Dahash and F.T. Juma, 1993. Effect of different diluents on Shami goats. Small Rumin. Res., 9: 347-352.

Bearden, H.L. and L.W. Fuquay, 1990. Applied Animal Reproduction Practice. INC., Englewood Cliffs, New Jersey.

Cross, N.L. and Meizel, 1989. Methods for evaluating the acrosomal status of mammalian sperm. Biol. Reprod., 41: 635-641.

Graham, J.K., 1996. Analysis of stallion semen and its relation on fertility. Veterinary Clin North Am. Equine Pract., 12: 119-129.

Gisen, A.f. and T.L. Sexton, 1983. Beltsville poultry semen extender 7. Comparison of commercial diluents for holding turkey semen 6 h at 15° C. Poult. Sci., 62: 379-3.

Hassan, M.M. and F.F. Graham, 1994. Carbonated Tris extender prolongs sperm life at 4°C storage. Pak. Vet. J., 14: 107-109.

Jainudeen, M.R. D. Santhana, 1982. Effect of level of glycerol rates of freezing and thawing on survival of buffalo spermatozoa in straws. Anim. Prod. Health Trop., 57: 409-411.

Johar, K.S., J.R. Lodge and W. Northon, 1976. A comparative study of several extenders bovine at room temperature. Indian J. Dairy Sci., 26: 188-191.

Libertado, C. Cruz., 1993. Reproductive biotechniques in water buffaloes. Philippine Carabao Center, Department of Agriculture, DCIEC Bldg, Quezon City, pp: 1-5.

Mohan, G. and K.L. Sahi, 1991. Comparative efficacy of certain extender containing various level of yolk for preservation of buffalo semen at 5 C?. Indian J. Anim. Sci., 61: 725-727.

Pursel, V.C., L.A. Johnson and L.L. Schulman, 1974. Acrosome morphology of boar spermatozoa during in vitro aging. J. Anim. Sci., 38: 113-116.

Rasul, Z., M. Anzar, S. Jalali and N. Ahmad, 2000. Effect of buffering system on post- thaw motion characteristics, plasma membrane integrity and acrosome morphology of buffalo spermatozoa. Anim. Reprod. Sci., 59: 31-41.

Sahni, K.L. and A. Roy, 1972. A note on the effect of twostorage temperature on the keeping quality of sheep and goat semen in different diluents. Indian J. Anim. Sci., 42: 580-583.

Sexton, T.J. and A.F. Giesen, 1982. Beltsville poultry semen extender 6. holding turkey semen for 6 h at 15° C. Poult. Sci., 61: 1202-1208.

Suzuki, H. and T. Niwa, 1981. Variation in intera-cellular enzyme, especially Glutamic Oxalaretic Transaminanse (GOT) and hyaluroidase. Bulletin of the laboratory of Artificial Insemination. Iwatas, University. N1, pp: 52-65.