ISSN 1819-1878

Asian Journal of Animal Sciences

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Asian Journal of Animal Sciences 6 (5): 249-255, 2012
ISSN 1819-1878 / DOI: 10.3923/ajas.2012.249.255

Effects of Extender Types on Ram Semen Collected with Electroejaculator in a Tropical Environment

K.M. Okukpe, A.A. Adeloye, K.D. Adeyemi, O.A. Olatunde, V. Ojo and F.E. Sola-Ojo
Reproductive Physiology Unit, Department of Animal Production, Faculty of Agriculture, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State, Nigeria

Corresponding Author: K.M. Okukpe, Reproductive Physiology Unit, Department of Animal Production, Faculty of Agriculture, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State, Nigeria Tel: +2348066716145

ABSTRACT

The aim of the study was to assess the effect of soymilk on spermatozoa and its use as an extender. Twelve Yankasa rams between 12-48 months old were used for the study. The semen was collected with an electroejaculator, evaluated for sperm concentration and percent sperm motility, live spermatozoa and sperm abnormalities. The best semen samples were randomly assigned to four extenders (Cornell University Extender, CUE), whole milk glycerol extender, soymilk glycerol extender and soymilk-CUE). These samples were stored for 4 days at 5°C and analyzed for semen quality. The result shows the differences between the extenders were not significant for the initial and final percent sperm motility, except for soymilk. There were no significant differences in the extenders for initial and final percent live-spermatozoa except in the spermatozoa extended in soymilk extenders. Out of the four extenders tested for semen preservation in this study, soymilk extender and soymilk-CUE least supported sperm motility and liveability, although soymilk-CUE was better than soymilk. The problem of the poor performance of the soymilk and soymilk-CUE could be due to the concentration of soybean fluid content and/or the antinutritional factors probably present in the soymilk due to the mode of preparation. Further studies are suggested along these lines.

Key words: Rams, semen, extender, soymilk, Cornell university extender, soybean fluid

INTRODUCTION

The success of Artificial Insemination (AI) particularly in cattle and sheep depends greatly on the development of satisfactory semen extenders which performs the same function as the seminal plasma. The seminal plasma secreted from the accessory reproductive organs serves as a transport medium for the spermatozoa (Kiyma *et al*., 2000; Boucif *et al*., 2011; Udeh *et al*., 2011). It also provides an environment with adequate pH for optimum metabolic activities. The contribution of the seminiferous tubules to the total semen volume is very small with the chemical composition of the semen varying with animal species and it is dependent on the relative contribution of the seminiferous tubules, vesicular glands, prostate gland and bulbourethral glands. The plasma serves as a buffered nutrient medium which suspends and maintains the fertility of spermatozoa (Gouletsou *et al*., 2003).

The seminal plasma is composed of a number of organic and inorganic compounds. The major organic ion found in seminal plasma is bicarbonate, produced by the vesicular glands and it functions as a buffering agent, regulating pH changes of semen (Hammerstedt, 1981;

Keskintepe *et al.*, 1998; Massanyi *et al.*, 2003). Plasma buffers are often not present in sufficient quantities to prevent a reduction in semen pH when in storage; therefore, a good semen extender should have buffering capacity (Okukpe *et al.*, 2001; Tajik *et al.*, 2007). Other organic compounds that serve primarily as energy substrate include fructose, sorbitol and Glycerylphosphorylcholine (GPC). Fructose (a simple sugar) and sorbitol (sugar alcohol) are produced by the vesicular glands while GPC is produced in the epididymis (Bearden and Fuquay, 1980; Zamboni, 1992; Arman *et al.*, 2006; Setchell, 2008).

Many extenders have been developed as a result of the discovery that sperm in whole semen lived for only short periods of time and that cooling whole semen very slowly to 5°C caused the death of many spermatozoa. Therefore, apart from increasing the ejaculate volume the extender protects the sperm during cooling as well as extends the life of the spermatozoa (Colas, 1983; Shakeri *et al.*, 2008; Kalaba and Abdel-Khalek, 2011). Some known extenders are skim milk-glycerol, Lactose egg-yolk-glycerol, egg yolk citrate, tris(hydroxymethyl) aminoethane-citric acid egg yolk glycerol, Illini Variable Temperature (IVT) extender, Cornell University extender (CUE), Tris-Coconut milk, Coconut milk-citrate, etc each with different preservation power and fertilization rate (Ahmed, 1989; Sekoni, 1990; Keskintepe *et al.*, 1998; Okukpe *et al.*, 2001; Preciado *et al.*, 2011; Kaplan *et al.*, 2011).

According to Keskintepe *et al.* (1998), skim milk glycerol extender gives higher fertilization rates than egg yolk-glycerol citrate extender post-thaw. Some of these extenders are of higher cost than others as they are not readily available locally. Soymilk in food value is comparable with cow milk and it is very easily digestible but has not been tested as a semen extender. This study was designed to evaluate the quality of spermatozoa in four different extenders-two established and two unknown extenders. These are Cornell University Extender (CUE), whole milk glycerol, soymilk glycerol and soymilk-CUE.

MATERIALS AND METHODS

The study was carried out at the small ruminant unit of the Federal University of Technology Akure, Teaching and Research farm. A total of twelve Yankasa rams were used for the study. The animals were managed under semi-intensive system. They were taken out for grazing (mixture of guinea and elephant grass in the morning after semen collection and later brought back in the afternoon for the second semen collection. No supplementary feed was given.

Processing of soymilk: The soybean sample (0.250 kg) was soaked for 18 h in deionized water (750 mL) to remove the bitter taste. It was later washed and coarsely ground twice with the addition of more water (500 mL). The slurry was well stirred and sieved. The soymilk was collected, heated to 90°C for about 10 min and refrigerated (Okukpe *et al.*, 2001).

Semen collection and evaluation: Semen collection was done by electroejaculation every other day for a period of 60 days covering the spermatogenic cycle (Bearden and Fuquay, 1980; Memon *et al.*, 1986; Oyeyemi *et al.*, 2001; Al-Ghalban *et al.*, 2004; Palmer, 2005; Sundararaman *et al.*, 2007a). The rams were ejaculated twice, morning (7.00-10.00 am) and afternoon (4.00-7.00 pm) on the day of semen collection. The semen was collected into graduated test-tubes in a flask containing warm water at 37°C. The semen was taken to the laboratory in this flask. In the Artificial Insemination (AI) laboratory, the semen samples were analysed for the following characteristics: semen volume, sperm concentration, sperm motility, live-dead ratio and

sperm abnormalities. The best semen samples randomly selected were preserved in the four different extenders (CUE, whole milk glycerol extender, soymilk glycerol extender and soymilk-CUE).

Semen extension: The semen samples in the graduated test-tubes were placed in a water bath maintained at 37°C for 30 min to equilibrate the temperature. Four milliliters of each extender was then divided into two equal parts of 2 mL each and glycerol was added to one part at twice the desired level. The semen and extenders (excluding glycerol) were then placed along with the water bath in the refrigerator and allowed to cool for two hours to about 5°C. Thereafter, 1 mL of semen was added to each of the extenders. The extenders containing glycerol at double strength was gradually added to the semen-extender mixture. This was done by dividing the extender plus glycerol into four parts and gradually adding one part to the mixture at 15 min interval. A dilution rate of 1 to 4 was used i.e., one milliliter of semen to four milliliters of extender. After processing, extended semen were packed in labeled bijou bottles and refrigerated for four days before it was re-evaluated for motility and live spermatozoa.

Semen volume was measured directly from the graduated test-tube but only used in concentration calculation due to established inconsistency of volume in electroejaculation method of collection. Sperm concentration was measured with a haemocytometer using the steps outlined by Sorensen (1979), Bearden and Fuquay (1980) and WHO (2004). Sperm motility was estimated by observing the movement of the spermatozoa under the x40 objective microscope (Sorensen, 1979). Semen smears stained with eosin-nigrosin (Bearden and Fuquay, 1980) were made for live-dead ratio and sperm abnormalities. The percentage of normal morphology was determined by using the uncleared slides (Osinowo *et al.*, 1982; Hafez and Hafez, 2000; Rodriguez-Martinez, 2003), under the microscope using x40 objectives.

Statistical analysis: All parametric data were subjected to Analysis of Variance (ANOVA) using MINITAB (V.10.1, Minitab Inc. USA) statistical package while comparisons between means were by Fisher's Least Significant Difference (LSD) (Steel and Torrie, 1980).

RESULTS

Extenders composition and effects of types of extenders on sperm motility and liveability are presented in Table 1 and 2, respectively. The mean initial percent motility for CUE, whole milk-

Table 1: Composition of the extenders

Extender components	CUE (g)	Cowmilk glycerol (g)	Soymilk glycerol (g)	Soymilk-CUE (g)
Sodium citrate dihydrate	14.50	-	-	14.50
Sodium bicarbonate	2.10	-	-	2.10
Potassium chloride	0.40	-	-	0.40
Glucose	3.00	0.5	0.5	3.00
Glycine	9.37	-	-	9.37
Sulphanilamide	3.00	-	-	3.00
Citric acid	0.87	-	-	0.87
Distilled water (mL)	1000.00	50.0	50.0	1000.00
Buffer (%)	80.00	3.0	3.0	80.00
Egg yolk (%)	20.00	10.0	10.0	-
Whole milk (%)	-	87.0	-	-
Soymilk (%)	-	-	87.0	20.00

CUE: Cornell university extender

Table 2: Effect of type of extender on sperm motility and liveability

	Motility (Mean±SEM, %)		Live-spermatozoa (Mean±SEM, %)	
Extenders	Initial (morning)	Final (afternoon)	Initial (morning)	Final (afternoon)
CUE	75.45±2.48	40.64±2.50[a]	81.89±2.70	44.89±2.89[a]
Whole-milk	73.69±3.86	40.06±4.10[a]	81.64±4.24	45.78±4.23[a]
Soymilk	64.89±6.18	22.25±3.62[b]	74.28±5.91	27.17±3.72[b]
Soymilk-CUE	64.72±7.08	36.06±5.33[a]	64.42±7.58	37.95±5.84[a]

CUE: Cornell university extender, Within column, means with different superscripts differ significantly at $p<0.05$

glycerol, soymilk-glycerol and soymilk-CUE were 75.45±2.48, 73.69±3.86, 64.89±6.18 and 64.72±7.08, respectively. Percent initial sperm motility with CUE and whole milk was close and higher than percent initial motility in soymilk and soymilk-CUE, though not significant. The mean final percent motility for CUE, whole milk, soymilk and soymilk-CUE were 40.64±2.50, 40.06±4.10, 22.25±3.62 and 36.06±5.33, respectively. Percent final motility with CUE, whole milk and soymilk-milk were close and were significantly ($p<0.05$) higher than percent final motility in soymilk.

The mean initial percent livability for CUE, whole milk, soymilk and soymilk-CUE were 81.89±2.70, 81.64±4.24, 74.28±5.91 and 69.42±7.58, respectively. Percent initial sperm livability with CUE and whole-milk was close and was higher than percent initial motility in soymilk and soymilk-CUE, though not significant. The mean final percent livability for CUE, whole-milk, soymilk and soymilk-CUE were 44.89±2.89, 45.78±4.23, 27.17±3.72 and 37.95±5.84, respectively. Percent final livability with CUE, whole milk and soymilk-CUE were close and were significantly ($p<0.05$) higher than percent final livability in soymilk.

DISCUSSION

It is evident from the semen characteristics presented in Table 2 that percent sperm motility and live-spermatozoa were higher in ram semen extended with Cornell University Extender (CUE), followed by whole milk extender, soymilk-CUE and soymilk extenders. This means that insemination of ewes with semen preserved in the first two extenders could favourably lead to effective fertilization and hence conception. This is in line with the reports of some earlier authors (Graham *et al*., 1978; Sekoni, 1990; Davis *et al*., 1993; Keskintepe *et al*., 1998; Gravance *et al*., 1998; Kishikawa *et al*., 2001; Padrick and Jaakma, 2002; Kikuchi *et al*., 2004; Esteso *et al*., 2006). It could be observed in Table 1 that CUE and soymilk-CUE contained the same proportion of buffering substances such as sodium citrate dehydrate, sodium bicarbonate, potassium chloride, glycine sulphanilamide and citric acid which are necessary for semen pH regulation (White, 1973; Bearden and Fuquay, 1980; Nehring, 2003; Kikuchi *et al*., 2004; Lambrechts *et al*., 2006), apart from their containing egg-yolk and soymilk, respectively. The difference in performance of the two extenders (CUE and soymilk-CUE) could be attributed to the presence of two different protein substances (egg-yolk and soymilk) albeit in the same proportion. Both extenders are rich in protein, with egg yolk containing about 16.15% protein and soymilk containing about 40% protein (Powrie and Nakai, 1986; Berk, 1992; Okukpe *et al*., 2001). However, they have varying essential and non-essential amino acids respectively. In addition to differing protein sources and therefore, amino acid mixtures, the poor performance of the soymilk extender therefore could be attributed to the presence of some traces of antinutritional factors such as trypsin inhibitor and lectin that were not inactivated by heat treatment, although, this factors was not investigated in this study. Antinutritional factors has been shown to inhibit metabolism (De-Jarnette *et al*., 1992; Aletor, 1993).

The enhanced performance of the whole milk and CUE in this study corroborates their performance in other studies (Foulkes *et al*., 1977; Muir, 1992; Keskintepe *et al*., 1998; Okukpe *et al*., 2001). As percentage of normal sperm morphology may be an indicator of the fertility potential of a given male (Sundararaman *et al*., 2007b). The poor performance of soy-CUE and soy-milk extenders compared to whole milk and CUE could be due to the nature of soymilk or the presence of residual antinutritional factors in the soymilk. Fertilizing potential of spermatozoa could be affected by period of storage (Shakeri *et al*., 2008; Dehghan *et al*., 2010). However, since the soymilk and soymilk-CUE extenders supports initial motility and liveability with deterioration after cold (5°C) storage for 4 days, further studies with improved processing methods ensuring complete removal of probable antinutritional agents is suggested for further studies in the use of soymilk as an extender agent.

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