EFFECT OF *GAMBAYA ALBIDA* JUICE ON THE SURVIVABILITY OF GOAT SEMEN UNDER STORAGE IN A TROPICAL ENVIRONMENT

I, Udeh, Akporhuarho, P.O* and Obushe, E.
Department of Animal Science, Delta State University, Asaba Campus, Nigeria.
*Corresponding author. E-mail:okpakophilip@yahoo.com.

Accepted 22nd July, 2017

Four healthy bucks of West African Dwarf goat certified free of andrological defects were used for the experiment to determine the survivability of goat sperm in *Gambaya albida* juice extender under two storage conditions, namely; refrigeration temperature (5°C) and room temperature (25°C). Four storage trials were carried out for the *G. albida* extender using semen collected from bucks by electron-ejaculation method. Statistical analysis showed highly significant (P<0.01), effect of storage days on the motility of buck semen. The highest mortality was maintained in the first 24 h of storage, thereafter, motility decreases as the storage days increases, At the fourth day of storage motility reduced drastically at room temperature while at room temperature motility score sharply dropped to zero value. The storage temperature was recorded to be highly significant (P<0.01), except on the 6th week of trial. The refrigeration temperature was superior compare to room temperature. The refrigeration temperature maintained sperm viability for a maximum period of 96 hr. The interaction between days and storage temperature showed was highly significant (P<0.01). The storage media was significant (P<0.05); except on the 4 and 6th week of trial. The result has been able to provide the usefulness of *G. albida* juice in formulating semen extender for both refrigeration temperature and room temperature.

Key words: Effect, *Gambaya Albida*, juice, survivability, goat and semen.

INTRODUCTION

Some of the challenges of goat production is to increase reproduction rates, various options of reproduction techniques that are available, among which are artificial insemination (A.I), and embryo transfer.

Artificial insemination can be defined as the act of injecting spermatozoa artificially into the vagina of female animal for the purpose of making or causing the animal to be pregnant (Iwena, 2008). It is often used in animal, to multiply possible offspring of a priced animal and for the breeding of enlarged species. It adds several quality and economic advantages for goat production. Prepared semen can be preserved for long period by using refrigeration, and it is frequently shipped over great distances.

Progress in semen collection and dilution and cryopreservation techniques now enables a single buck to be used simultaneously in several countries for up to 100,000 inseminations a year (Gibson and Smith, 1989). Several researches have been conducted in developed countries to determine the best media and storage condition for buck semen (Lopez et al., 2000)

Gambaya albida is a fleshy, smooth, drupe fruit having a single seed and a weak groove. It is native to other Northern Hemisphere and flourishes well in a temperature climate. It is believed that the Roman discovered the sweet cherry fruit in the Asia Minor in about 70BC. They then introduced them in the first century AD to Britain and the genus prunus, also shares some traits with peaches, almonds, apricots and plums. The fruit is cultivated in different varieties like the wild cherry, sweet cherry and the tart cherry. The fruit is mainly grown for consumption, and its well-known for its delicious and healthy juice.

In the Nigeria context, some plant materials such as pawpaw, coconut, and tomatoes have been used in the formulation of semen diluents. The present study is designed to find out the suitability of *G. albida* juice as a constituent

of goat semen. Therefore, the research is aimed at determining the effect of fresh *G. albida* juice and storage day on the motility of goat semen under room and refrigeration temperature.

MATERIALS AND METHODS

Location of the study

The experiment was undertaken at the laboratory of the Department of Animal Science, Delta State University, Asaba Campus. The annual rainfall of Asaba campus ranged from 200 to 2177mm, most of which falls between April and October, and dry season from November to March.

Experimental animals

Four west African dwarf goats (WAD bucks) were used for the experiment. The four bucks were kept in a well illuminated pen. They were semi-intensively managed in clean, well ventilated pen with a concrete floor. The bucks were about 2 years old with an average body weight of 17.15 kg, and were fed grass such as guinea grass *(panicium maximum*), elephant grass (*pennisetum purpureum*), and carpet grass (*Axonopus compressors*) in the morning, and concentrate supplement in the evening. They were served clean fresh water *ad libitum*. Routine medication consisted of deworming with Albendazol (Phemix, Belgium) at a dosage of 2ml/16 kg body mass and vaccination against Peste des petite ruminants (PPR) using tissue culture rinder pest vaccine (NVRI, VOM, Nigeria).

Materials used

The materials used for the experiment includes microscope, P^H meter, slide, cover slips, electro ejaculator, distilled water, tri-sodium citrate, eggs, gambaya albida, egg separator, centrifuge, penicillin and streptomycin, volumetric flask, needles, syringes and oven.

Procedures

Preparations of buffer

2.9 g of Tri-sodium citrate were weighed. Freshly boiled distilled water was added to 2.9 g of tri sodium citrate in a 100 ml volumetric flask. The mixed solution of citrate and distilled water was put in the dark cupboard until the solution was cooled, and some distilled water was added to make up 100 ml.

Preparation of G. albida juice

The *G. albida* fruits used for the experiment were procured from the market, it was properly washed to remove all the dirt in it, and the head was opened and the juice extracted from it. The juice was stored in a clean sterilized container and kept in the refrigerator until use.

Preparation of diluents

The buffer was measured with a measuring cylinder of 100 ml. Egg yolk was separated from a freshly broken egg, and put into a small beaker. The *G. albida* juice was warmed for 5 min, and allowed to cool. The required amounts (Table 1) of *G. Albida* juice, egg yolk, buffer, penicillin and streptomycin were measured into a measuring cylinder and well shaken; the resultant mixture was poured into centrifuge tubes for centrifugation. P^H was measured using P^H meter. The diluents were kept in a refrigerator until ready for use in about an hour or above.

Table 1. Relative amounts of constituents of diluents**.**

Variable	Buffer (ml)	Egg yolk (ml)	*G. albida* juice (ml)	Antibiotics
Treatment 1	90	5	5	1 ml each
Treatment 2	80	10	10	1 ml each
Treatment 3	70	20	15	1 ml each
Treatment 4	70	10	20	1 ml each

All the aforementioned constituents of dilutes were prepared in the same day.

Collection of semen

Semen collection was done once a week by electro – ejaculator method (Oyeyemi, 2001). All aseptic protocols were observed during semen collection. The electro – ejaculator probe was lubricated with petroleum jelly (Vaseline) before insertion into the buck's rectum. The electro-ejaculator was plugged to a

source of electricity and switched on. Two attendants held the buck firmly in a standing position. Voltage was applied to the animal by rotating the knob of the variable resistor, turning it back to zero, and repeating the process several times by gradually increasing the applied voltage, the nerves responsible for ejaculation was stimulated and erection took place followed by ejaculation. With a clean warn sterilized funnel and semen graduated tube (bathed in warm water at 37°C), semen was collected from the buck and stored at 37°C in water bath until extended. Precaution taken before semen collection includes:

1. Adequate restraint in a crutch
2. Cleaning of prepuptial area with clean water and towel
3. Trimming long and dirty prepuptial hairs

Processing of semen

Immediately after semen collection, a drop of semen was put on clean warn glass slide, and then covered with cover slip and placed under microscope to assess the progressive motility. Progressively motile sperm cells are those ones which move in forward direction with rapid swirling wave motion. Motility was secured immediately after extension. Prior to semen extension, volume was quickly determined from a graduated collecting tube and the values recorded. The vials were kept in a refrigerator temperature at 5°C, and room temperature at 25°C. The colour of the semen was determined by visual assessment. The semen colour was creamy and normal with high density. Sperm movement was assessed 24 hourly basis for 4 days.

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA), using 4 x 4 x 2 factorial in a completely randomized design. The means and standard error of the means were calculated for each parameter (Steel and Torrie, 1980). Duncan multiple range test (Duncan, 1955) was used to separate the means where ANOVA showed significant effect.

RESULT

It will be observed from Table 2, that percentage motility of buck spermatozoa decreased from day 1 to 4 throughout the 8 weeks period of the experiment. The highest motility was obtained in day 1. This was followed by day 2, 3 and 4 in that order.

Table 2. Means and standard error of effects of storage days on goat sperm motility (%) measurement of error.

Weeks of collection	Storage days			
	D1	D2	D3	D4
1	80.00±0.00^{d}	33.13±3.77^{c}	14.38±3.20^{b}	2.50±0.94^{a}
2	80.63±6.25^{d}	27.50±5.90^{c}	12.50±3.53^{b}	6.25±2.45^{a}
3	90.00±0.00^{d}	38.13±4.53^{c}	10.00±2.99^{b}	2.50±0.94^{a}
4	85.00 ±0.00^{d}	30.00±4.82^{c}	11.88±4.11^{b}	6.25±2.45^{a}
5	80.00 ±0.00^{d}	26.25±3.63^{c}	11.25±2.45^{b}	3.75±1.57^{a}
6	80.00 ±0.00^{d}	32.50±3.89^{c}	12.50±3.89^{b}	6.25±2.45^{a}
7	90.00 ±0.00^{d}	30.00±6.68^{c}	12.50±3.89^{b}	3.75±1.57^{a}
8	80.00 ±0.00^{d}	31.25±5.40^{c}	12.50±3.89^{b}	5.00±2.31^{a}

a,b,c and d means different superscripts letters along each row are highly significant (P<0.01).

Means and standard error

The results shown in Table 3 presents the effect of storage media on the motility of bucks sperm cells. It was observed that T1 maintained the highest motility in almost the whole weeks of the experiment except on the 5 and 7th weeks where the highest motility was found in T2 and T4, respectively.

Table 4 present the means and standard errors for the sperm motility of buck's sperm cell stored at room temperature and refrigeration temperature. It will be observed that refrigeration temperature gave the highest sperm motility compared to room temperature. This may be as a result of the cooling effect of refrigeration temperature on the sperm cells.

Table 3. Means and standard error effect of storage media on the motility of bucks spermatozoa.

Weeks of collection	Storage media			
	T1	T2	T3	T4
1	34.38±11.20[b]	33.13±11.22[a]	33.13±11.45[a]	31.25±11.52[a]
2	35.63±11.43[b]	30.63±11.59	30.00±11.80[a]	30.63±11.67[a]
3	36.25±13.05[b]	34.38±13.44[a]	35.00±13.32[b]	35.00±13.23[b]
4	34.38±12.08	32.50±12.28	33.13±12.42	28.27±11.64
5	34.38±11.55[b]	31.88±11.34[b]	31.25±11.45[b]	28.27±11.64[a]
6	34.38±11.08	34.38±11.08	31.25±11.56	32.50±11.26
7	35.75±13.22[b]	34.38±13.41[ab]	32.50±13.56[a]	35.63±13.04[b]
8	34.48±11.28[b]	30.00±11.84[a]	30.00±11.84[a]	34.38±11.28[b]

From each now, means with different superscripts letters are significantly (P<0.05) different .

Table 4. Means and standard error for sperm motility of bucks stored at room and refrigeration temperature (%).

Weeks	Room temperature	Refrigeration temperature
1	27.50±8.16[a]	37.50±7.22[b]
2	24.38±8.60[a]	39.06±6.75[b]
3	29.69±9.38[a]	40.63±8.50[b]
4	25.94±8.99[a]	0.63±7.20[b]
5	25.63±8.28[a]	35.00±7.22[b]
6	26.25±8.34[a]	39.38±6.69[b]
7	26.25±9.59[a]	41.88±8.09[b]
8	25.00±8.39[a]	39.38±6.94[b]

a<b (P<0.01).

From the result presented in Table 5, it was observed that the highest motility was maintained in day 1 in both room temperature and refrigeration temperature, however, refrigeration temperature is preferred, because motility was sustained up to 50.00% of the initial motility compared to room temperature.

DISCUSSION

In Table 2, it will be observed that the percentage motility of buck spermatozoa decreased from day 1 to 4 throughout the 8 weeks period of the experiment. The highest motility was obtained in week 1, this was followed by day 2, 3, and 4 in that order. This result is in agreement with the observation of Igboeli (1970) and Peterson et al. (2007) who reported that semen stored in liquid form begin to decrease progressively 24 h after storage, and a better result is obtained when used for Artificial insemination.

The cause of death for the sperm cells could be as a result of depletion of sperms intrinsic and extrinsic energy sources. Sperm motility enhances energy depletion. Amann (1970) reported the spermatozoa survived best in a highly concentrated state when their motility is reduced to a minimum as in the epididymis.

Table 3 present the effect of storage media on the motility of bucks sperm cells. It will be observed that T1 maintained the highest motility at weeks 1,2,3,5,7 and 8 followed closely T4 in weeks 7 and 8. This could be attributed to factors such as impurities and microorganisms that affect the viability of sperm cells (Lafalci et al, 2002)

The observation of this study is that type of extender that affected the maximum survival time of buck semen which is in line with the report of Nwike (1994) who reported that type of extender that affected the maximum survival time of boar semen.

In Table 4, the highest motility was maintained under the refrigeration temperature (5°C) compared to that of room temperature (25°C) throughout the 8 weeks of trials. This is because of the cooling effect produced during storage at refrigeration temperature, this cooling effect, help to stop metabolic process of stored semen which result in utilization of nutrient such as fructose (Agboagla, 2003).

It will be observed from Table 5, that spermatozoa stored in refrigeration temperature (5°C) maintained the highest motility score

Table 5. Means and standard error of storage temperature by storage day's interaction on the motility of bucks spermatozoa.

Weeks collection	Room temperature				Refrigeration temperature			
	1	2	3	4	1	2	3	4
1	80.00^{e} (0)	23.75^{c} (2.39)	6.25^{b} (1.25)	0^{a} (0)	80.00^{e} (0)	42.50^{d} (1.44)	22.50^{c} (1.44)	5.00^{ab} (0)
2	81.25^{c} (1.25	12.50^{b} (6.25)	3.75^{a} (2.39)	0^{a} (0)	80.00^{e} (0)	42.50^{d} (2.50)	21.25^{e} (1.25)	12.50^{b} (2.16)
3	90.00^{f} (0)	26.25^{d} (1.25)	2.50^{ab} (1.44)	0^{a} (0)	90.00^{f} (0)	50.00^{e} (0)	17.50^{e} (1.44)	3.00^{b} (0)
4	85.00^{f} (0)	17.50^{c} (1.44)	1.25^{a} (1.25)	0^{a} (0)	85.00^{f} (0)	42.50^{e} (1.44)	22.50^{d} (1.44)	12.50^{b} (2.16)
5	80.00^{e} (0)	17.50^{c} (1.44)	5.00^{ab} (0)	0^{a} (0)	80.00^{e} (0)	35.00^{d} (2.89)	17.50^{c} (1.44)	7.50^{b} (1.44)
6	80.00^{e} (0)	22.50^{c} (1.44)	2.50^{a} (1.44)	0^{a} (0)	80.00^{e} (0)	42.50^{d} (1.44)	22.50^{c} (1.44)	12.50^{b} (1.44)
7	90.00^{f} (0)	12.50^{c} (1.44)	2.50^{a} (1.44)	0^{a} (0)	90.00^{f} (0)	47.50^{e} (1.44)	22.50^{d} (1.44)	7.50^{b} (1.44)
8	80.00^{f} (0)	17.50^{cd} (1.44)	2.5^{ab} (1.44)	0^{a} (0)	80.00^{f} (0)	45.00^{e} (2.89)	22.50^{d} (1.44)	11.25^{c} (2.89)

a,b,c,d,e,f, means along the same row are significance (P<0.01) different.

throughout the 8 weeks of trials. Although both room temperature (25°C) and refrigeration temperature were viable to maintain sperm viability for the first 24 h of storage but refrigeration temperature exceeded 24 h of storage. The reason for this observation could be as a result of cooling effects which tends to inhabit the growth of micro-organisms that is produced during heat, thereby maintaining the viability of sperm cells (Lafalci *et* al., 2002). Other factors that may be responsible for the significant variation observed in the motility of sperm cells are; initial semen quality before storage, processing methods and composition of the advent (Osinowo, 2006).

CONCLUSION AND RECOMMENDATION

From the results obtained in this study, it could be concluded that *G. albida* juice extender in semen storage does not adversely affect sperm survival. Further studies could be carried out on the use of other fruits such as mango juice,and so on.

Another conclusion drown from this study is that diluted liquid semen stored at 5°C should be kept beyond 2 days in order to obtain a good conception rate when used for insemination. Also, from the result obtained. Storing buck semen at refrigeration temperature was better compared to room temperature.

Based on the result obtained in this study, it is recommended for anybody who desires to experiment with semen extender to use *G. albida* juice, this is because *G. albida* juice does not have any adverse effect on spermatozoa survival. And it should be used immediately after extraction for better result and to prevent the accumulation of toxic chemical such as lactic acid which may hinder the survival rate of sperm cells. It is also advisable to use refrigeration temperature which is the best storage medium of buck semen.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Agboagla, E.M., and Terada, T. (2003). Trehalose – enhanced fluidity of the goat sperm membrane and its protection during freezing. Boil. Reprod. 69:1245-1250.

Amann, R.P. (1970). "Sperm Production Rates" in the test is A.D. Johnson, W.R. Gomes and N.L. Van Demark (ed), Academic Press, New York, pp. 433-482.

Igboeli, E. (1970). A Tris buffered egg yolk extender for boar semen. Int. J. Anim. Sci., 30(2):569-572.

Iwena, O.A. (2008). Essential Agricultural Science 5th edition.

Gibson, J.P., and Smith, C. (1989). The in corporation of biotechnologies into animal breeding strategies. In: Babiuk L.A, Philips J.P and Moo Young M. (eds); Animal Biotechnology. Comprehensive Biotechnology first supplement. Pergamon Press, Oxford, UK pp. 2003-231.

Oyeyemi, M.O, Akusa, M.O, and Ola – Davies, O.E. (2001). effect of successive ejaculation on the spermiogram of West African Dwarf goats. Israel Vet. Med. J. 56(4)

Steel, R.G.D., and Torrie, J.H. (1980). principles and procedures of statistics: Biometrical approach, 2nd edition, McGraw Itili, New York. Duncan Multiple Range Test.

Peterson, K. (2007). Microscopic and floe cytometric semen assessment of Dutch A.I bucks; Effects of semen processing procedure and their correlation to fertility.

Lafalci, V.S., Tortorella, H., Roctrigues, J.I., and Bran aelli., A. (2002). Seasonal variation of goat seminal plasma proteins. Theriogenology (57): 1035 – 1048.

Lopez, S.A., Ortiz, N., Gallego, L., and Garde, J.J. (2000). Liquid storage (5^0C) of ram semen in different diluets. Arch. Androl., 44(2): 155.

Osinowo, O.A. (2006). Introduction to Animal reproduction Sophine Academic pp. 74-75.