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ORIGINAL RESEARCH ARTICLE

Effects of Heat-Treated *Aloe barbadensis* Miller Leaf Gel on Quality and Spermatozoa Fertilizing Potential of Extended Red Sokoto Goat Semen

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ABSTRACT

This study aims at investigating the Heat-treated *Aloe barbadensis* leaf gel effects on quality and spermatozoa fertilizing potential of extended red Sokoto goat buck semen**.** *Aloe barbadensis* gel was scooped, homogenised, filtered, heat-treated at 60°C ($Gel_{60°C}$) and stored at 4°C for 72 hours. It was used to replace glycerol as a cytoprotectant in Egg Yolk+Sodium Citrate (EYSC) diluent at 0.0, 1.75, 3.50, 5.25, and 7.00g/L. The diluent was used to extend semen samples from three red Sokoto bucks (2-3 years old) weighing 41.5±2.0kg and stored at –22°C. Extended semen samples were evaluated at 0, 24, 48, and 72 hours for quality and spermatozoa fertilizing potential. All treatments were replicated thrice in a completely randomized design. Progressive Motility (PM), Spermatozoa Livability (SL), Normal Spermatozoa (NS), Acrosome Integrity (AcI), Plasma Membrane Integrity (PMI), and Secondary Morphological Abnormalities (SMA) were evaluated at 24-hour intervals for 72 hours. Data were analyzed using descriptive statistics and ANOVA at $\alpha_{0.05}$. PM, NS and AcI respectively ranged from 58.33-68.33%, 83.67-89.00% and 81.67-92.67% which were significantly higher than 56.67%, 77.67% and 83.33% in the control. $Gel_{60°C}$ sustained the quality of extended buck semen similar to the control up to 72 hours of storage. Also, $Gel_{60°C}$ added to diluent at 7.00g/L offered cytoprotectant to extended buck spermatozoa at 72 hours.

Keywords: Red Sokoto goat, Semen extender, spermatozoa quality, cytoprotectant

Introduction

Semen for Artificial Insemination (AI) could be collected fresh and inseminated immediately or collected, extended, and inseminated fresh or preserved for future use. Semen preservation or storage could be for a short, medium, or long time depending on storage conditions. The possibility of semen transportation across borders due to freezing has reduced the barriers to breeding animals (Veerkamp and Beerda, 2007). Preserving cells or tissues by chilling them in liquid nitrogen at extremely low temperatures is a method for long term storage of semen. Freezing is done in industry, medicine, and nanotechnology to extend storage life of specific components indefinitely using molecules called cytoprotectant such as glycerol. Semen kept at temperatures between 5 to 8°C survives for 1 to 2 days with insignificant loss of motility and up to 4 days without drop in fertilization potential (Batellier *et al.*, 2002). Although refrigeration provides a convenient way for short-time semen preservation, yet it has lethal effects on the quality of the spermatozoa. It reduces viability, motility, fertilising potentials and increases morphological abnormalities (Batellier *et al.*, 2002; Medeiros *et al.*, 2002). Spermatozoa can be stored indefinitely for future use without significant reduction in fertilization rates post-thaw when stored at -196 ℃ in liquid nitrogen (Morrell, 2011). However, scarcity of liquid nitrogen in Nigeria as well as other developing and underdeveloped countries necessitate the use of deep freezing to preserve semen for more than 48 hours. Cyto-preservative offers protection from cold shock and structural damage during freezing for spermatozoa. However, glycerol is somewhat toxic to spermatozoa (Holt, 2000) and may induce osmotic damage (Purdy, 2006). Its addition may cause certain structural damage and lowered motility of spermatozoa (Salamon and Maxwell, 2000; Sonmez and Dimirci, 2004). The use of natural extract from fruits, vegetables, and their seeds in extenders for preserving animal semen was introduced for their protective properties in preserving sperm cells (Sansone *et al.*, 2000). *Aloe vera* has been classified as medicinal plant with specific bio-active ingredients such as alkaloids, saponins, flavonoids, proteins, lipids, amino acids, vitamins C, B (1, 2 and 6), A, E, enzymes, organic and inorganic compounds, and mineral salts such as sodium, calcium, iron, potassium, chloride, manganese, copper, and zinc (Yamaguchi, *et al.*, 1998). It has been used as a natural antioxidant with high

potential of reducing fat oxidation and oxidative stresses (Vinson *et al.,* 2005). Pharmacological effects of Aloe vera include anti-inflammatory, anti-arthritic, and antimicrobial (Newall *et al*., 1996). For semen preservation, Aloe vera appears as an alternative to vegetal origin, as it contains some compounds that can act as conventional cytoprotectants (Boudreau and Beland 2006). In recent times, Aloe vera has been used in several studies which show the positive action of Aloe vera extract on spermatogenesis (Prerna, *et al*, 2024; Mohammad, *et al*., 2018; Mohammad, *et al.,* 2014*;* Niknam, *et al.,* 2014 Estakhr and Javdan, 2011). Therefore, it presents a potentially viable alternative for spermatozoa cytoprotection and should be thoroughly investigated and explored. Also, Souza *et al*., (2016), using Aloe vera as a cryoprotectant in peccaries sperm extender, observed that sperm motility, viability and membrane integrity values were similar to egg yolk-based extender. In another study by Agbaye *et al*., (2023) it was observed that raw gels of *Aloe barbadensis* maintained plasma membrane integrity of spermatozoa in extended buck semen above 60% after 48 hours. However, very few documented studies on the effects of *Aloe vera* the leaf extracts on spermatozoa characteristics have been reported.

MATERIALS AND METHODS

Experimental Site

Semen collection was done at the Small Ruminant Unit of the University of Ibadan Teaching and Research Farm, located at ($7^{o}20^{1}$N, $3^{o}50^{1}$E; 200m above sea level), while the semen quality and spermatozoa fertilizing potential were evaluated at the Animal Physiology Laboratory, Department of Animal Science, University of Ibadan, Nigeria.

Preparation of Heat-Treated *Aloe barbadensis* Miller Leaf Extract

Freshly harvested and authenticated *Aloe vera* leaves were washed. The gel was carefully scooped with a spatula into a beaker, homogenized and heated in a water bath at a temperature of 60°C for 30 minutes. The heat-treated gel was allowed to cool to room temperature and refrigerated at -1°C. This product is referred to as Heat Treated *Aloe barbadensis* Gel ($Gel_{60°C}$).

Semen Processing

Three mature and healthy Red Sokoto bucks aged 2-3 years, weighing 41.5 ± 2.0 kg were ejaculated weekly using electro-ejaculation method as described by Oyeyemi *et al*. (2000). Ejaculates were collected into calibrated tubes using a funnel.

Semen Extender and Experimental Layout

Treatment	Composition
T_1	7.00 (GEYCE) + 0.00 ($Gel_{60°C}$)
T_2	5.25 (GEYCE) + 1.75 ($Gel_{60°C}$)
T_3	3.50 (GEYCE) + 3.50 ($Gel_{60°C}$)
T_4	1.75 (GEYCE) + 5.25 ($Gel_{60°C}$)
T_5	0.00 (GEYCE) + 7.00 ($Gel_{60°C}$)

$Gel_{60°C}$ = Heat-treated (60°C) *Aloe barbadensis* gel; GEYCE = glucose-egg yolk-citrate extender

RESULTS

Table 1. Characteristics of fresh ejaculate of Red Sokoto buck used for the study

Parameter	Value
Semen Volume (mL)	0.75 ± 0.24
pH	6.60 ± 0.18
Progressive Motility (%)	95.00 ± 2.06
Spermatozoa Livability (%)	99.00 ± 1.50
Normal Spermatozoa (%)	98.00 ± 2.25
Acrosome Integrity (%)	98.00 ± 3.19

Effect of $Gel_{60°C}$ on quality and spermatozoa fertilizing potential in extended buck semen at 0Hr

The effect of heat-treated *Aloe vera* leaf extract ($Gel_{60°C}$) on characteristics of extended buck semen at zero hour is shown in Table 2.

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Table 2: Effect of $Gel_{60°C}$ on Quality and Spermatozoa Fertilizing Potential in Extended Buck Semen at 0Hr

Parameter	$Gel_{60°C}$ (g/L)					
	0.00	**1.75**	**3.50**	**5.25**	**7.00**	**SEM**
PM (%)	90.00	88.33	90.00	88.33	85.00	0.59
SL (%)	91.67	88.33	86.67	83.33	90.00	0.93
NS (%)	91.67	90.00	88.33	86.67	88.33	0.55
Ph	6.73^{a}	6.50c	6.60^{b}	6.63^{b}	6.60^{b}	0.02
AcI (%)	90.00^{a}	81.67^{b}	88.33^{a}	88.33^{a}	88.33^{a}	0.94
PMI (%)	91.67^{a}	83.33^{b}	83.33^{b}	86.67^{ab}	86.67^{ab}	0.99

^{ab} = In a row, values having different alphabet differ statistically ($P<0.05$), SEM is Standard Error of Means
PM=Progressive Motility, SL=Spermatozoa Livability, NS=Normal Spermatozoa, AcI=Acrosome Integrity, PMI=Plasma Membrane Integrity.

Table 3: Effect of $Gel_{60°C}$ on Quality and Spermatozoa Fertilizing Potential in Extended Buck Semen at 24 Hours (-22°C)

Parameter	$Gel_{60°C}$ (g/L)					
	0.00	1.75	3.50	5.25	7.00	SEM
PM (%)	63.33	58.33	59.67	63.33	63.33	1.48
SL (%)	87.67	88.00	86.00	82.00	85.67	2.15
NS (%)	90.00^{a}	81.00^{b}	88.00^{a}	85.67^{a}	77.00^{b}	1.57
pH	6.77^{b}	6.57^{c}	6.50^{c}	6.73^{b}	6.90^{a}	0.05
AcI (%)	90.33^{a}	85.67^{a}	74.67^{b}	73.67^{b}	76.67^{b}	2.13
PMI (%)	85.00^{a}	81.67^{a}	74.67^{b}	75.33^{b}	76.33^{b}	1.30

^{ab} =In a row, values having different alphabet differ statistically (P<0.05), SEM= standard error of means, PM= Progressive Motility, SL=Spermatozoa Livability, NS=Normal Spermatozoa, AcI=Acrosome Integrity, PMI=Plasma Membrane Integrity

There was no significant difference (p>0.05) in PM, SL, and NS in semen samples extended with $Gel_{60°C}$ before freezing. However, pH in the control was higher significantly (p<0.05) than in all samples extended in extender with $Gel_{60°C}$. Acrosome integrity is relatively (p<0.05) higher in the control than in samples extended with 1.75 g/L of $Gel_{60°C}$ but similar to other treatments. Plasma membrane integrity is 14.34 % higher in samples extended with 0.00 g/L of $Gel_{60°C}$ than those in 1.75 and 3.5 g/L of $Gel_{60°C}$. However, all recorded values for all treatments are good enough to achieve fertility if used for insemination.

Effect of $Gel_{60°C}$ on Quality and Spermatozoa Fertilizing Potential in Extended (-22°C) Buck Semen at 24 Hours

The effects of $Gel_{60°C}$ on buck semen parameters after 24 hours of freezing at -22°C is shown in Table 3. The PM in all the treatments decreased appreciably ranging from 51.7 ± 2.9 to 63.3 ± 10.4 % compared with 85.0 ± 5.0 to 90.0 ± 0.0 % recorded at 0 hour. There was no statistical (p>0.05) difference in PM and SL across the treatments. The samples extended with 0.00 g/L $Gel_{60°C}$ had significantly (p<0.05) higher AcI and PMI values than all $Gel_{60°C}$ treatments except those extended in 1.75 g/L $Gel_{60°C}$. The NS were 13 % and 9 % higher in sample preserved in 0.00 g/L of $Gel_{60°C}$ compared with samples in 7.00 and 1.75g/L of $Gel_{60°C}$ respectively. Except for samples in 3.50 g/L of $Gel_{60°C}$, pH varied significantly at 24 hours compared to 0 hour with samples in 7.00 g/L of $Gel_{60°C}$ significantly (p<0.05) higher than others.

Effect of $Gel_{60°C}$ on Spermatozoa Quality and Spermatozoa Fertilizing Potential in Extended Buck Semen at 48 Hours (-22°C)

The effects of varying concentration of $Gel_{60°C}$ at 48 hours post extension and storage is shown in Table 4. shows significant (p<0.05) difference in PM in all semen samples extended and frozen both with the varying concentration of the extract and the control although all values were higher than the required 60% for successful insemination. pH in samples in 5.25 g/L of $Gel_{60°C}$ was 10 % higher than those in 0.00 g/L of $Gel_{60°}$. The SL was significantly (p< 0.05) higher in 0.00 g/L of $Gel_{60°C}$ compared to other treatments and about 20 % higher than samples in 7.00 g/L of $Gel_{60°C}$. The NS for samples preserved with 5.25 mL and 7.0 mL of the extract respectively were similar to the control while the samples preserved with 1.75 and 3.50 g/L of $Gel_{60°C}$ had significantly lower values (p<0.05) than the control.

Table **Error! No text of specified style in document.**: Effect of $Gel_{60°C}$ on Quality and Spermatozoa Fertilizing Potential in Extended Buck Semen at 48 Hours (-22°C)

Parameters	$Gel_{60°C}$ (g/L)					
	0.00	1.75	3.50	5.25	7.00	SEM
PM (%)	75.00^{b}	73.33^{b}	61.67^{c}	85.00^{a}	63.33^{c}	2.74
SL (%)	86.67^{a}	80.00^{b}	83.33^{b}	83.33^{b}	66.67^{c}	2.36
NS (%)	81.67^{a}	60.00^{c}	76.67^{b}	86.67^{a}	86.67^{a}	3.19
pH	6.73^{a}	6.50^{c}	6.60^{b}	6.63^{b}	6.60^{b}	0.04
AcI (%)	95.00^{a}	95.00^{a}	93.33^{a}	96.67^{a}	80.00^{b}	1.97
PMI (%)	68.00^{a}	65.00^{ab}	71.00^{a}	64.33^{ab}	64.00^{ab}	0,86

^{ab} = In a row, values having different alphabet differ statistically (P<0.05), SEM is Standard Error of Means
PM=Progressive Motility, SL=Spermatozoa Livability, NS=Normal Spermatozoa, AcI=Acrosome Integrity, PMI=Plasma Membrane Integrity.

Table 5: Effect of $Gel_{60°C}$ on Quality and Spermatozoa Fertilizing Potential in Extended Buck Semen at 72 Hours (-22 °C)

Parameter	$Gel_{60°C}$ (g/L)					
	0.00	1.75	3.50	5.25	7.00	SEM
PM (%)	56.67[b]	58.33[b]	66.67[a]	63.33[a]	68.33[a]	1.47
SL (%)	85.00	84.33	84.33	80.00	86.33	0.69
NS (%)	77.67[b]	88.33[a]	89.00[a]	85.67[a]	83.67[a]	1.31
pH	6.63[b]	6.40[c]	6.87[a]	6.50[c]	6.67[b]	0.05
AcI (%)	83.33[ab]	81.67[b]	92.67[a]	86.00[ab]	87.33[ab]	1.22
PMI (%)	77.00	73.33	73.33	73.67	73.33	0.46

[abc] =Values on the same row carrying different superscripts differ statistically (P<0.05), SEM is Standard Error of Means. PM=Progressive Motility, SL=Spermatozoa Livability, NS=Normal Spermatozoa, AcI=Acrosome Integrity, PMI=Plasma Membrane Integrity.

The AcI was statistically (p<0.05) lower (80.0 ± 10.0 %) in sample extended with 7.0 mL compared to others including those in 0.00 g/L of $Gel_{60°C}$ while treatment containing 5.25 mL of extract recorded the highest value (96.7 ± 5.8 %).

Effect of $Gel_{60°C}$ on Quality and Spermatozoa Fertilizing Potential in Extended Buck Semen at 72 Hours (-22°C)

Table 5 shows that samples frozen in extenders with concentration of 3.50 g/L $Gel_{60°C}$ and above had significantly (p>0.05) higher PM than the control. Samples in 7.00 g/L of $Gel_{60°C}$ also recorded highest SL but it is not significantly (p<0.05) different from other samples extended in $Gel_{60°C}$. The results also demonstrated that all of the $Gel_{60°C}$ containing treatments for NS had significantly higher values (p>0.05) than the control. Spermatozoa in semen samples extended in 3.50 g/L of $Gel_{60°C}$ extenders were statistically (p>0.05) higher in pH, NS and AcI values than others.

DISCUSSION

Impact of $Gel_{60°C}$ on Spermatozoa Quality and Fertilizing Potential in Extended Buck Semen stored at -22°C

The characteristics of extended buck semen shows a significant (p>0.05) slight reduction in the quality of the extended buck semen sample compares to fresh ejaculate. This result is similar to the findings of several studies (Okukpe *et al*., 2012, Jun-He Hu, *et al*., 2013 and Iman *et al.,* 2015). However, this reduction was not significant for PM, SL and NS in samples extended with $Gel_{60°C}$ compared to those extended with glycerol extender before freezing. The significant reduction in quality could be linked to the effects of heat on some bioactive components of the extract. Solton *et al.,* (2016) reported 69.70 % PM, 77.70 % SL, 85.90 % AcI, and 78.00 % PMI for fresh buck semen extended in an extender containing 15% soybean milk which is lower than values obtained in this study. George *et al*. (2009), reported that *Aloe vera* extracts should not be exposed to high temperatures or filtered which may destroy or reduce the effects of certain essential enzymes and polysaccharides. After 24 hours of freezing, the post-thawed semen characteristics of buck semen samples extended with extenders containing a varying concentration of $Gel_{60°C}$ shows a significant reduction in PM compared to fresh semen and pre-freeze samples. This could be due to the gradual depletion of nutrients required for high metabolic demands for spermatozoa transport. Ottaway (2010) reported significant losses of vitamin C and thiamine when fruits and vegetables are heated during processing. When heated at 65°C for less than 15 minutes, the biological activity of *Aloe vera* gel remains intact, however longer time or higher temperatures have been found to drastically lower its activity levels. At 70°C, *aloe vera* polysaccharide is most stable, but it degrades at both higher and lower temperatures (Xiu *et al.,* 2006). Antoni *et al.*, (2003) reported that physical and chemical alterations occur in acemannan, a bioactive polysaccharide in Aloe vera parenchyma as a result of heat treatment and dehydration at temperatures 30 to 80°C. The bioactive polysaccharide's average molecular weight increased from 45 kDa in fresh aloe to 75 kDa in samples heated to 70-80°C. The physicochemical alterations of the main type of polysaccharide may have important implications on the physiological activities attributed to the *Aloe vera* plant. However, in this study, despite the seeming loss of some ingredients in the $Gel_{60°C}$ (51.67±2.9 to 63.33±7.6) % obtained for PM still fall within 50.00 to 70.00% PM regarded as good motility by Johnson *et al.* (2003) to achieve excellent fertility rate. $Gel_{60°C}$ preserved the quality of buck semen up to 72 hours of freezing which is an indication that $Gel_{60°C}$ offers anti-freezing effects thereby preventing or reducing intra-cellular ice formation which is detrimental to the cellular membrane. Post-thawed PM after 72 hours of freezing still ranged between 58.33±7.6 to 68.33±2.9% in samples frozen in $Gel_{60°C}$ which was

higher than 56.67±5.8% obtained in samples frozen in glycerol extender. Also, $Gel_{60°C}$ recorded over 80.00±0.0% of SL with the intact plasma membrane, apical acrosome integrity and were still morphologically normal. This is an indication that $Gel_{60°C}$ preserves to a large extent the morphological structure and fertilizing potential of extended buck semen frozen at -22 ℃ up to 72 hours of freezing.

CONCLUSION

Heat-treated *Aloe barbadensis* gel added to diluent at 7.00g/L offered cytoprotective ability to extended buck spermatozoa to a tolerable level up to 72 hours.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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