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The Preservative Effect of Pineapple Juice on Chilled Bull Semen

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Abstract

This study was conducted to determine the preservative effects of pineapple juice on the progressive motility (PM) and plasma membrane integrity (PMI) of chilled bull semen. Semen samples obtained from bred Holstein bulls were diluted in egg yolk citrate extender (EYC) and firstly substituted with pineapple juice (*Ananas comosus*) at four levels of substitution; 0.25ml, 0.50ml,0.75ml, and 1ml of pineapple juice (PJ) in five treatments; T2, T3, T4, and T5 respectively with T1 being the control. Secondly, pineapple juice (*Ananas comosus*) was added at four levels of addition; 0.25ml, 0.50ml,0.75ml, and 1ml of pineapple juice (PJ) in five treatments; T2, T3, T4, and T5 respectively with T1 being the control. The diluted semen samples were preserved at a temperature of 5^0C and microscopic evaluation was done each day for PMI and PM until all the sperm cells died. The results obtained indicate a higher progressive motility and plasma membrane integrity for both the substitution and addition of pineapple juice in the EYC extender compared to the control. It was therefore evident that the substitution of egg yolk in the EYC extender with pineapple juice at 25% led to higher PMI and PM. Substitution of egg yolk in egg yolk citrate extender at 75% and 50% did not show a significant difference with the control while a 100% substitution resulted in a rapid drop in PMI and PM values. There was a higher ($P<0.05$) PMI and PM at all levels of addition as compared to the control with the highest PMI and PM obtained from T2, T3, T4, and T5 but shows no statistically significant difference ($P<0.05$) between them. It is therefore evident pineapple juice in the EYC extender has a significant positive effect on plasma membrane integrity as compared to the control with no pineapple juice.

Keywords: Bull semen, Pineapple, Progressive motility, Plasma membrane integrity

Introduction

Cattle's rearing which is the principal sector of the animal industry plays an undeniable socio-economic role in Cameroon's economy. About 10.36% of the gross domestic product (GDP) of the private sector comes from animal rearing (1), from which over 61.2% is from cattle which registers 5-6 million cattle head (2). Three regions found in the Sudano Sahelian zones of the country (Adamawa, North, and Far North regions) contribute 83% of the total cattle production and 17% of this production is from the North West, South East and East regions of the national territory.

It has been reported that the annual demand for milk in Cameroon is estimated at 297000tons. This demand cannot be made because our local breeds have very low productivity in terms of milk (3). There is, therefore, a need to look for alternative ways to upgrade our indigenous cattle in terms of milk production, to meet up with the growing demands for these products in Cameroon and other neighboring African countries.

This can be achieved through the application of modern production techniques such as artificial insemination (AI) which has been widely accepted by local farmers to boost production and genetic improvement. AI has been a major step in attaining the objectives of improving cattle production and productivity in Cameroon (4). The success of AI depends on many factors among which are the qualities of extender. The fertilizing capacity of sperm depends on the maintenance of viability, motility, and fertility of sperm cells during storage (5).

In the past, extenders such as EYC have been widely used, however; the duration of viability of this extender is limited. Munsi *et al.* (6) reported that chilled bull semen could be preserved for 5 days and retained 50% of post-storage progressive motility only. It is therefore necessary to identify alternative means of extending the motility of spermatozoa for a longer period. Pineapple is rich in health-promoting antioxidants such as ascorbic acid, flavonoids, and other phenolic compounds related to antioxidant activity as stated by Redal *et al. (*7) and Mhatre, (8) which is also rich in energy sources such as glucose, fructose (9) can be effective if used in extending bull semen.

The aim of this work was therefore to evaluate the effects of pineapple juice (PJ) used in egg yolk-citrate extender on the progressive motility (PM) and plasma membrane integrity (PMI) during liquid storage at 5^0C of bull semen.

Material and methods

Description of the study site

This study was carried out at IRAD Bambui regional center

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located in Tubah subdivision, Mezam division, North West region of Cameroon. The center is situated at latitude 6º North and longitude 10º and an altitude of 1600m above sea level. According to the ecological zones of Cameroon, IRAD Bambui is found in the Western highlands of Cameroon and is characterized by two seasons, a dry season and a rainy season with an average annual rainfall of about 2230mm uniformly distributed from mid-march to mid-November. The other months are virtually void of rain. The average maximum and minimum temperatures are respectively 25^0C and 14^0C. The average relative humidity is 52% in the dry season and 70% in the rainy season (10).

Biological material and their management

Animals

Semen used in this experiment was collected from a pure mature Holstein bull aged 7 years. The animal received a daily ration of 4kg of protein-based concentrate and grass.

Eggs

Eggs used for these experiments were layers of eggs bought from the polyvalent research station of the Institute of Agricultural Research for Development (IRAD) Mankon and stored in a refrigerator.

Semen collection

Semen samples were obtained from the Holstein bull using an artificial vagina. Ejaculates with motility above 75% were used for the present study. The semen collected was immediately taken to the laboratory for evaluation, processing, and extension. Each treatment was done in eight replicates giving a 5 x 8 factorial design.

Preparation of pineapple juice

A ripe pineapple of 1.5kg was bought at Nkwen Bamenda food market (Mezam division). The pineapple was washed with clean running tap water four times and rinsed twice with distilled water. The pineapple was peeled using a sterile knife and chopped into small pieces. It was ground using a mechanical grinder and it was further put in a clean sterile napkin for squeezing and the juice obtained was centrifuged at 2000rpm for 15 minutes. The supernatant was collected and the pH was measured. The juice was then put in sterile tubes and kept in the refrigerator for further use.

Preparation of egg yolk-citrate extender

The extenders used were prepared based on the egg yolk-citrate extenders following the protocol presented at IRAD Bambui and the proportion of eggs was first substituted (Table 1) and secondly added with pineapple juice at 4 levels as indicated in Table 2.

Post-extension evaluation of the semen

Motility and plasma membrane integrity were the 2 parameters taken into consideration during this study. The motility was evaluated by looking at a drop of diluted semen under a light microscope at magnification 400X. For cell integrity, it was evaluated using the HOS test (11). Evaluation of these parameters was done daily until all cells died.

Statistical analysis

The effects of substituting egg yolk with pineapple juice in egg yolk-citrate extender on progressive motility and plasma membrane integrity on sperm cells were evaluated using Sigma Stat graphics 4.0 and an analytical package. One-way ANOVA analysis was used to test differences among factors. The relationship between the various factors was done using a linear regression analysis. An angular transformation of data was done to render it more uniform. Fisher's LSD test was used to separate treatment means. Levels of significance were considered at $P<0.05$.

Results

Effects of substitution of egg yolk with pineapple juice (PJ) in egg yolk-citrate extender (EYC) on PM and PMI

From Table 3 below, we can see that varying the level of substitution of egg yolk in EYC extender with 75%, 50%, and 0% of pineapple juice did not significantly differ ($p<0.05$) on their effect on PM and PMI. Substituting egg yolk with 25% of PJ significantly differ ($p<0.05$) from other treatments and led to higher PM and PMI as compared to other treatments. While substitution of egg yolk with 100% pineapple juice in egg yolk citrate extender shows the least results as it resulted in lower PM and PMI values.

Effect of substitution of egg yolk with pineapple juice (PJ) in egg yolk-citrate extender and preservation duration on PM and PMI

Results in Table 4 indicate that the substitution of egg yolk with pineapple juice at 25 % had a significant ($p<0.05$) effect on both progressive motility and plasma membrane integrity during the whole preservation period. However increasing the level of substitution at 50% (T3), and 75% (T4) had no significant effect ($P>0.05$) on both PM and PMI under refrigeration conditions during the first week. Substitution at 50% (T3) and 75% (T4), did not have any significant difference ($P>0.05$) with the control (T1) in both parameters during the second week. Nevertheless, the complete substitution of egg yolk with pineapple juice (T5) in egg yolk citrate extender (EYC) was not favorable as it led to the lowest progressive motility and plasma membrane integrity values which were significantly lower ($p<0.05$) than the values obtained from the control experiment. It is therefore evident that the substitution of egg yolk in the EYC extender with pineapple at 25% led to higher plasma membrane integrity. Substitution of egg yolk in egg yolk citrate extender at 75%, 50% did not show a significant difference with the control. When the egg yolk was substituted at 100% we observed a rapid drop in plasma membrane integrity.

Effect of substitution of egg yolk with PJ in EYC extender on progressive motility of sperm cells with duration in storage

Figure 1 shows a decrease in progressive motility with time from the day of extension still the end of an experiment where motility reached zero. We also observe a sharp decrease in progressive motility of treatment T5 until it reaches zero at day 7. Meanwhile, will the treatment T1 (control) as compare to other treatments, until day 7, the progressive motility was still at about 65% before dropping sharply to 31.75% at day 9 and decrease progressively until it reaches zero at day 18. Treatment T2 decreases gradually until day 8 where the progressive motility was still almost 50%.

We also observe that diluents maintain progressive motility of 50% up to 7 days with treatment T1 and T4.

Effect of substitution of egg yolk with PJ in EYC extender on the evolution of plasma membrane integrity of sperm cells with duration in storage

From Figure 2, we can observe a gradual decrease in the plasma membrane integrity from the day of dilution up to day 21 with a sharp decrease on treatment 5 (T5) up to day 9 where it reaches 0. Treatment 2 (T2) has a gentle slope as compared to all the other treatments.

Effects of addition (supplementation) of egg yolk with pineapple juice (PJ) in egg yolk-citrate extender (EYC) on PM and PMI

Table 5 shows that varying the levels of addition of pineapple juice in the EYC extender from 25% to 50% and to 75% levels did not significantly ($p<0.05$) differ. But adding pineapple juice to the EYC extender greatly improved the plasma membrane integrity proven by a significant difference between the control with no pineapple juice added on one hand and 25% to 50% and to 75% on the other hand.

This result (Table 5) also reveals that varying the levels of addition of pineapple juice in the EYC extender has a significant effect on the motility of chilled bull semen this is demonstrated by a significant difference between the control (T1) and the other treatments containing pineapple at various levels. It is also observed that the best effect of pineapple juice in plasma membrane integrity was observed from treatment 4 (T4) containing 0.50ml of pineapple juice who is denoted by an (a) followed by the treatment4 containing 75% of pineapple juice though no significant difference to treatment 3 (T2) and 4 (T4).

Table 6 indicates that varying the levels of addition of pineapple juice in the EYC extender has a significant effect ($p<0.05$) on the motility and plasma membrane integrity of chilled bull semen. It is also observed that variation in the level of addition of pineapple juice from 25% to 50% and to 75% did not have any significant ($p >0.05$) effect on the progressive motility throughout the experiment. The best effect of pineapple juice in progressive motility was observed from treatment 3 containing 0.50ml (50%) of pineapple juice.

The results (Table 6) also reveal that adding pineapple juice to the EYC extender greatly improved the plasma membrane integrity proven by a significant difference ($p<0.05$) between the control (T1) on one hand and 25% (T2), 50% (T3), 75% (T4) and 100% (T4) levels of addition on the other hand. However, varying the levels of addition of pineapple juice in the EYC extender from 25% to 75% levels did not significantly affect ($p>0.05$) the plasma membrane integrity of bull sperm cells under refrigeration conditions.

Effect of supplementation of egg yolk with PJ in EYC extender on progressive motility of sperm cells with duration in storage

From Figure 3, we observe that progressive motility decreases with time from the day of dilution down to the end of an experiment where they all reach almost zero. We also observe that treatments T1 and T5 have the same trend from the day of dilution up to day 5 where the progressive motility is still above 70%. Treatments T2, T3, and T4 have almost the same trend throughout the experiment with T_3 showing a better performance. We also observe that diluents maintain progressive motility of 50% up to 13 days with treatment T2, up to 7 days with treatment T1, up to 13 days with treatment $T3$, up to 11 days with treatment T4, and up to 9 days with treatment T5.

Effect of supplementation of egg yolk with PJ in EYC extender on the evolution of plasma membrane integrity of sperm cells with duration in storage

Figure 4 reveals that, on the day of dilution, the plasma membrane integrity of sperm cells in all the treatments is almost the same. We also observe that treatments T2, T3, T4, and T5 have the same tendency as compared to treatment T_1 with preservation time. We also notice that treatments T2, T_3, and T4 perform better as compared to T5 and T1.

Table 1. Substitution of proportion egg yolk in EYC with pineapple juice (PJ) in extender

Level of egg yolk substitution	Citrate salt (g)	Penicillin (g)	Streptomycin (g)	Egg yolk (ml)	pineapple juice (ml)	Distilled H_2O (ml)	Total (ml)
0% PJ: T1	0.294	0.00750	0.01250	2.5	0	10	12.52
25% PJ: T2	0.294	0.00750	0.01250	1.875	0.625	10	12.52
50% PJ: T3	0.294	0.00750	0.01250	1.25	1.25	10	12.52
75% PJ: T4	0.294	0.00750	0.01250	0.625	1.875	10	12.52
100%PJ:T5	0.294	0.00750	0.01250	0	2.5	10	12.52

The various salt, Citrate salt, penicillin, and streptomycin sulfate were each weighed using a sensitive electronic scale and dissolved in distilled water as shown in Table 1 above.

Table 2. Addition of pineapple juice to the EYC extender

Level of egg yolk addition	Citrate salt (g)	Penicillin (g)	Streptomycin (g)	Egg yolk	Pineapple juice (ml)	Distilled H_2O (ml)	Total (m)
0% PJ: T1	0.294	0.0075	0.0125	2.50	0	10	12.5
25% PJ: T2	0.294	0.0075	0.0125	2.50	0.25	10	12.75
50% PJ: T3	0.294	0.0075	0.0125	2.50	0.5	10	13
75% PJ: T4	0.294	0.0075	0.0125	2.50	0.75	10	13.25
100%PJ:T5	0.294	0.0075	0.0125	2.50	1.00	10	13.5

Table 3. Effect of substitution of egg with pineapple juice in EYC extender on progressive motility (PM) and plasma membrane integrity (PMI) bull semen.

Level of egg yolk substitution with PJ	Means (%)	
	PM	PMI
100% PJ: T5	13.11c	29.53c
75% PJ: T4	31.94b	66.25b
0% PJ: T1	33.71b	67.15b
50% PJ: T3	34.07b	70.01b
25%PJ:T2	39.75a	83.12a

All means have the same standard error of ± 2.99 for plasma membrane integrity and 1.51 for progressive motility; a, b, c: least square means with the same superscript in the same column do not significantly differ from each other at (p<0.05).

Table 4. Effects of substitution of pineapple juice (PJ) in EYC extender on semen parameters in storage

Treatments	Preservation period (Days)					
	1-7		8-14		15-21	
	PM	PMI	PM	PMI	PM	PMI
0% PJ: T1	80.68±4.42a	80.29±4.35a	19.75±3.84b	19.98±3.94b	0.74±0.93b	0.96±1.08b
25% PJ: T2	78.89±4.42a	80.41±4.35a	35.54±3.84a	38.21±3.94a	4.82±0.93a	6.05±1.08a
50% PJ: T3	.89±4.42a	77.12±4.35a	25.14±3.84ab	26.28±3.94b	1.18±0.93b	1.65±1.08b
75% PJ: T4	71.67±4.42a	73.50±4.35a	22.14±3.84b	23.85±3.94b	1.71±0.93b	2.02±1.08b
100%PJ: T5	39.11±4.42b	43.89±4.35b	00.21±3.84c	0.50±3.94c	0.00±0.93b	0.00±1.08b

a, b, c: least square means with the same superscript in the same column do not significantly differ from each other at (P<0.05)

Table 5. Effect of supplementation of egg with pineapple juice in EYC extender on progressive motility (PM) and plasma membrane integrity (PMI) bull semen

Level of egg yolk substitution with PJ	Means (%)	
	PM	PMI
0% PJ: T1	33.13d	67.67c
100% PJ: T5	42.40c	87.88b
25% PJ: T2	49.68b	90.56a
50% PJ: T3	53.88a	92.02a
75%PJ:T4	52.96ab	96.92a

all means have the same standard error of 2.57 for plasma membrane integrity and 1.21 for progressive motility; a, b, c: least square means with the same superscript in the same column do not significantly differ from each other at (p<0.05).

Table 6. Effects of supplementation of pineapple juice (PJ) in EYC extender on semen parameters in storage

Treatments	Preservation period (Days)					
	1-7		8-14		15-21	
	PM	PMI	PM	PMI	PM	PMI
0% PJ: T1	79.07±2.36c	59.14±5.01c	19.96±3.91c	22.61±8.46c	0.36±1.97c	0.25±4.66c
25% PJ: T2	86.00±2.36ab	73.86±5.01ab	55.36±3.91a	31.07±8.46a	7.68±1.97ab	0.75±4.66bc
50% PJ: T3	90.64±2.36a	86.0±5.01ab	60.4 3±3.91a	40.71±8.46a	10.57±1.97a	0.36±4.66ab
75% PJ: T4	91.32±2.36a	83.29±5.01a	54.93±3.91a	90.54±8.46a	12.64±1.97a	0.93±4.66ab
100%PJ: T5	82.50±2.36bc	67.36±5.01bc	40.07±3.91b	83.82±8.46b	4.64±1.97bc	0.26±4.66a

a, *b, c: least square means with the same superscript in the same column do not significantly differ from each other at (P<0.05)*

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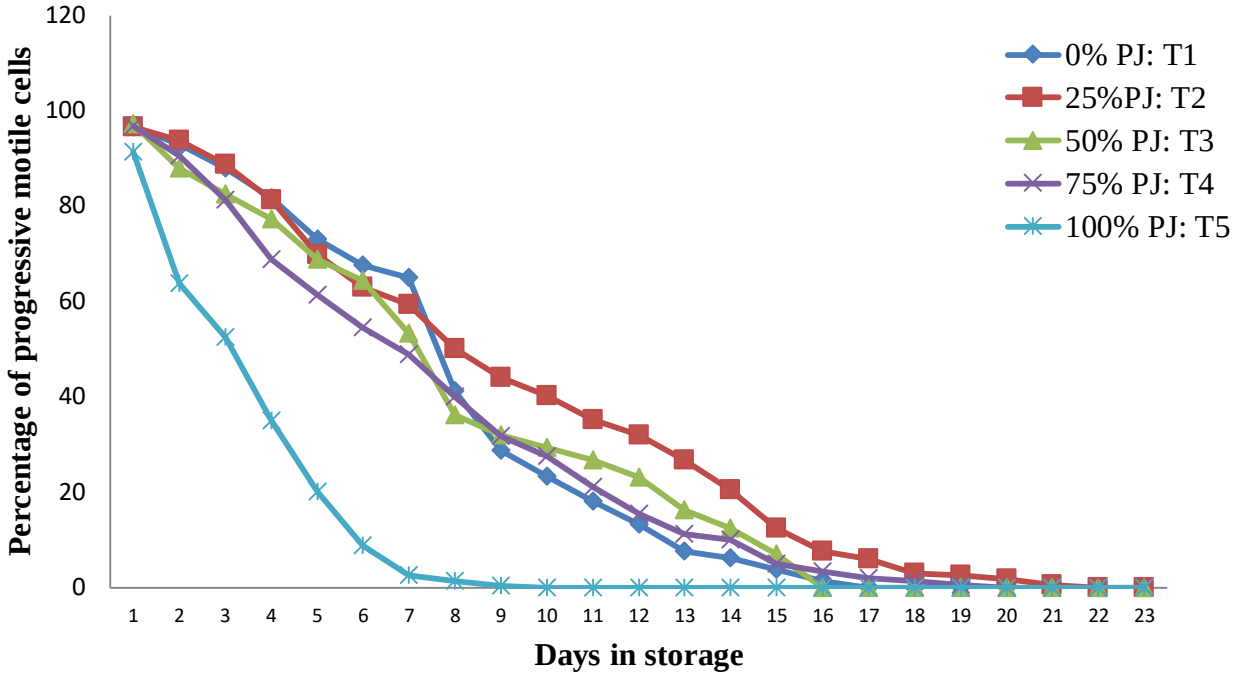

Figure 1. Evolution of percentage of progressive motile cells in extenders with duration in storage

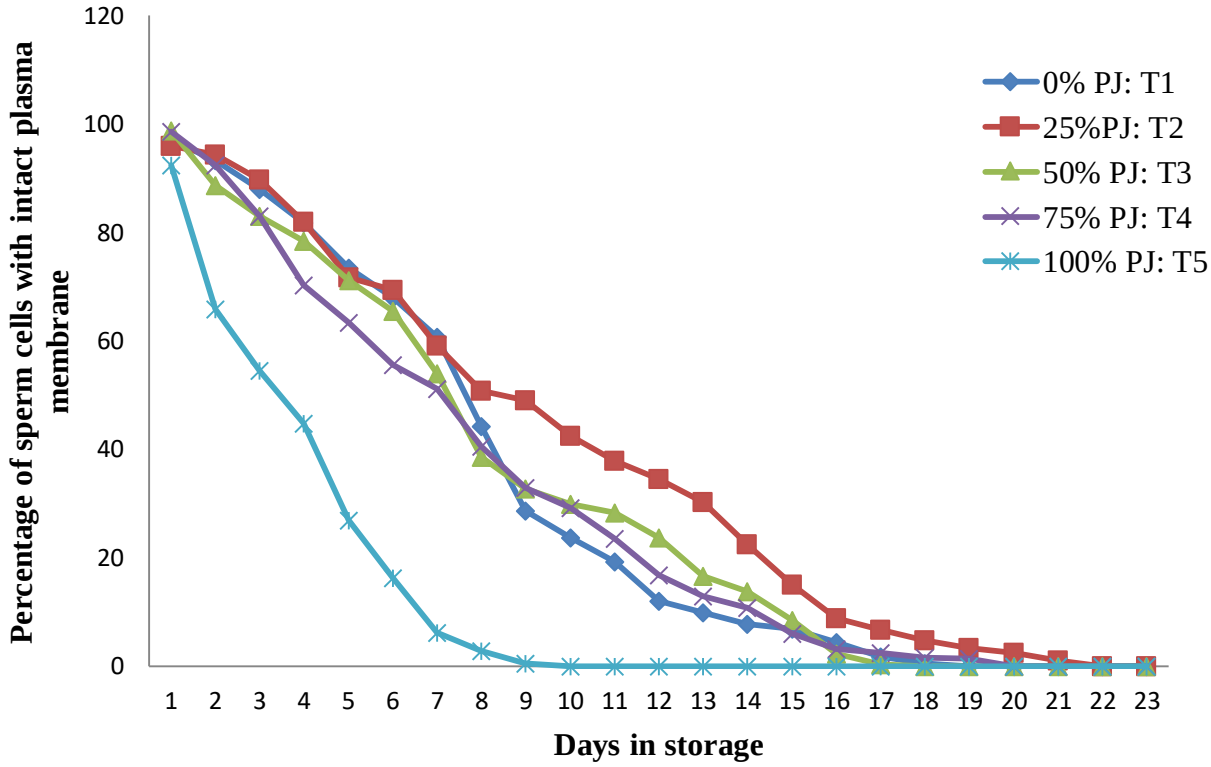

Figure 2. Evolution of percentage of cells with intact plasma membranes in extenders with duration in storage

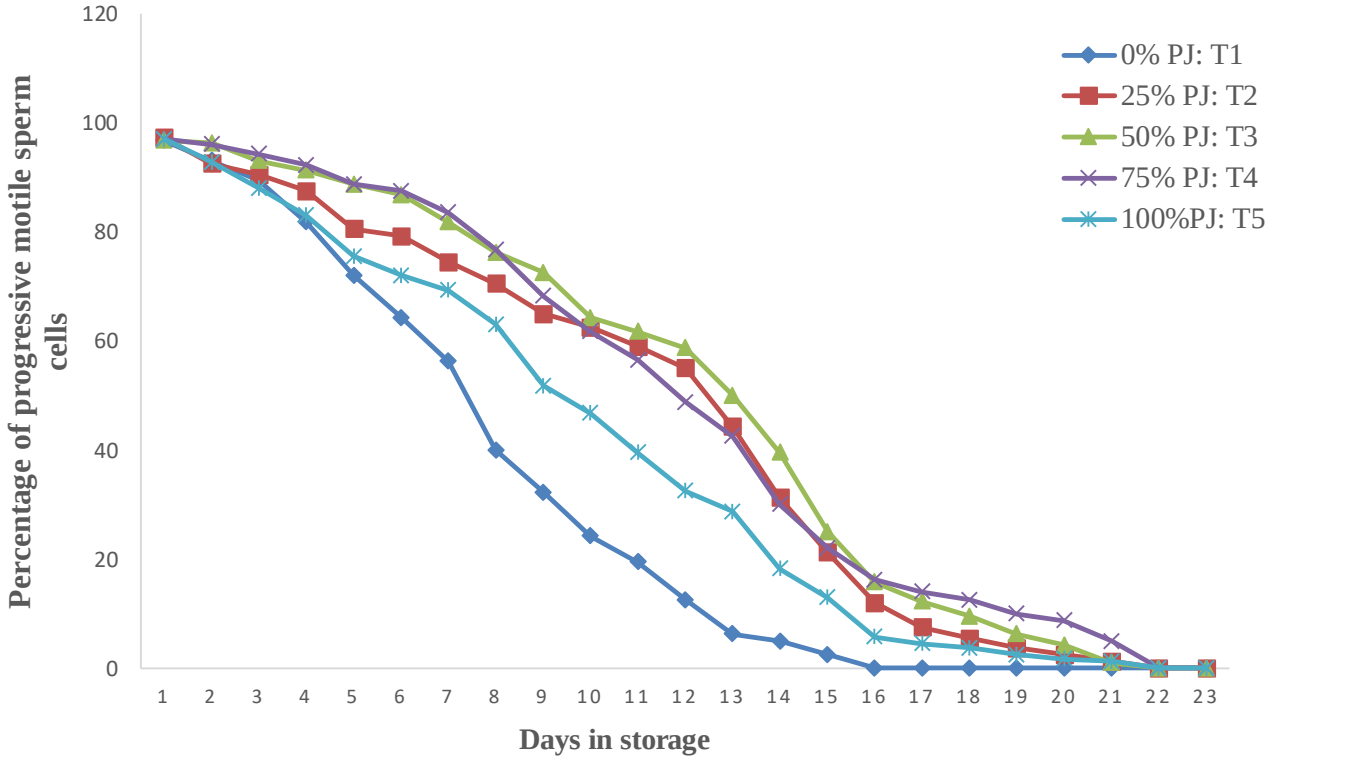

Figure 3. Evolution of percentage of progressive motile cells in extenders with duration in storage

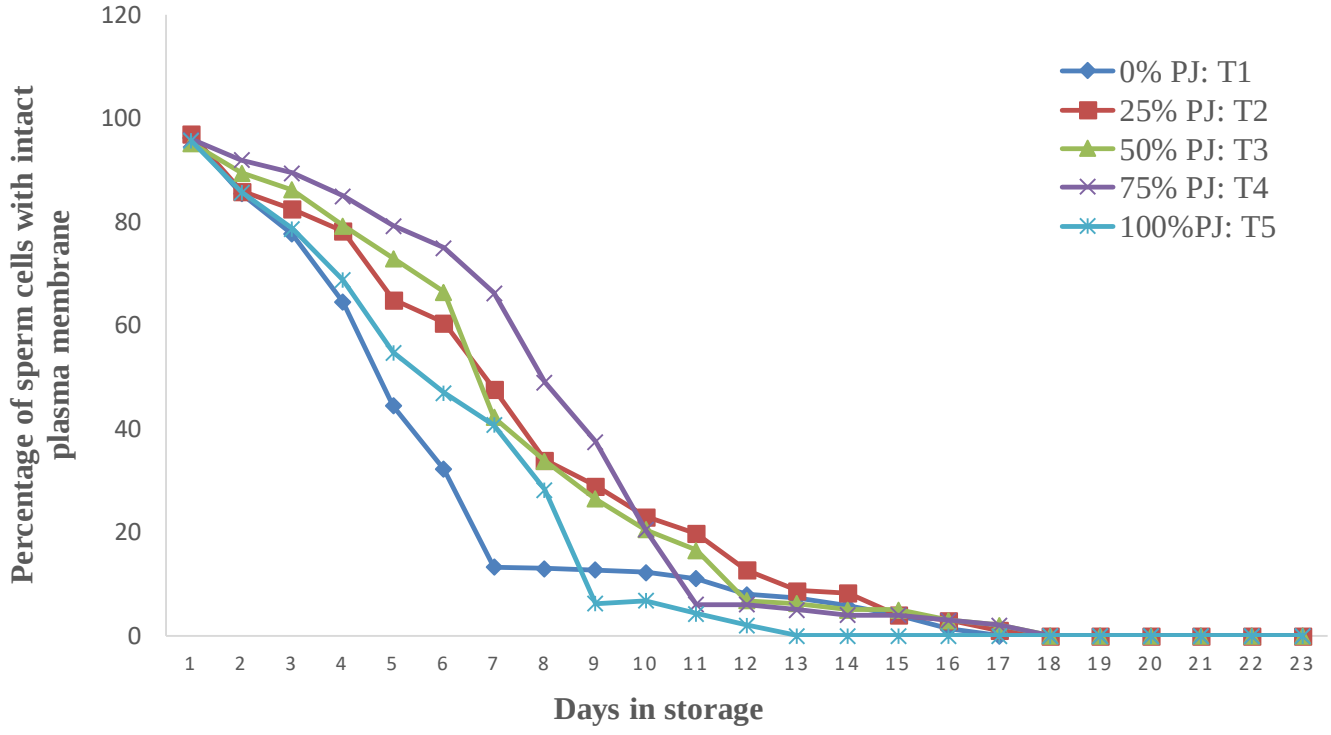

Figure 4. Evolution of percentage of cells with intact plasma membranes in extenders with duration in storage

Discussion

The results obtained indicate a higher progressive motility and plasma membrane integrity for both the substitution and addition of pineapple juice in the EYC extender compared to the control. This is in conformation with the work of Daramola *et al.* (12) who found that chilled semen of West African dwarf buck supplemented with pineapple juice showed higher progressive motility and plasma membrane integrity as compared to the control. The inclusion of pineapple juice in the EYC extender for cold storage of semen showed that this fruit juice can sustain progressive motility. This could be because, pineapple is rich in vitamins C, and E, and another health-promoting antioxidants such as ascorbic acid and flavonoids (7, 8). Reza *et al.* (13) showed that vitamin E or C supplementation in chilled stored semen improved the motility of spermatozoa. The antioxidant capacity of the pineapple juice might not however be due to vitamin C only but could also come from some phenolic compounds present in these fruits. Phenolic compounds neutralize free radicals known as superoxide, hydroxyl radical and nitric oxide and in addition act synergistically with other antioxidants, giving them extra potency to reduce free radical damage to cell membranes (14). The higher motility registered in this study at the various pineapple juice levels could therefore be due to the concentration of vitamins and phenolic compounds as earlier reported by Zuo *et al.* (14). Pineapple is rich in glucose and fructose (9, 15), and sperm cells utilize these sugars for respiration. Sugars also provide osmotic balance and cryoprotection for sperm cells (16).

In the present study, the substitution of egg yolk did not have a very significant effect on the preservation characteristics of chilled preserved Holstein bull semen as compared to the control except at 25% (T2) substitution; but the addition of pineapple juice in this study had a greater effect on sperm cells as compared to the control (T1). Since semen with a progressive motility of 30% can be used for AI (17), the results of the present study show that semen in extenders with pineapple juice addition can be used up to 14 days for treatments 2, 3, and 4 and up to 12 days for treatment 5.

Conclusion

Our findings revealed that extenders supplemented with pineapple juice significantly improved motility, and membrane integrity as compared to the control extender. Only a 25% substitution of egg yolk by pineapple juice was significantly different from the control. Total substitution of egg yolk with pineapple juice led to poor sperm motility and plasma membrane integrity compared to the control. It is therefore evident that adding pineapple juice to the EYC extender has a significant positive effect on plasma membrane integrity as compared to the control with no pineapple juice.

Recommendations

Based on the results obtained from this study, semen extenders should be supplemented with pineapple juice since it is an energy source and an antioxidant capable to prolong the viability and the potential fertilizing capacity of sperm cells. Also, research on in vitro and in vivo fertilizing capacity of semen extended with pineapple juice should be investigated.

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Conflict of interest

The authors declare no conflict of interest in the realization and publication of this piece of work.

Authors contributions

Hakoueu Flora designed the proposal and supervised the laboratory works, data collection, and manuscript writing. Mbiba Hassanu, Pierrette Ngo Bahebeck, Ndzi Elvis Ndukong. Nsadzetsen Gilbert Adzemye and Isabelle Leinyuy were in charge of laboratory analyses and manuscript realization.

Ethical issue

This study is under the ethical guidelines of the Institute of Agricultural Research for Development (IRAD), Cameroon.

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