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Spermatozoa survival and fertility of Nera black breeder semen extended with carrot juice

***Ewuola E.O, Osanyinlusi T. M, Ogundeji K.T and Oyedele D. M**

Animal Physiology and Bioclimatology unit,
Department of Animal Science, University of Ibadan, Ibadan, Nigeria

****Corresponding Author:** eoewuola@gmail.com;* ***Phone Number:** +2348060862361*

***Target Audience:** Poultry Breeders, Animal Physiologists and Veterinarians*

Abstract

This experiment was conducted to examine the fertilising ability of layer breeder cock spermatozoa extended with normal saline fortified with varying levels of carrot juice using 10 breeder Bovan Nera cocks. Semen was collected from the cocks, pooled and divided into six portions (treatments). Treatment 1(T_{US}) was undiluted semen which served as a control, while Treatments 2 (T_{NS}) and 3 (T_{MRS}) were diluted at 1:1 with normal saline and modified ringer solution, respectively. Treatments 4 ($T_{25\%CJ}$), 5 ($T_{50\%CJ}$), and 6 ($T_{75\%CJ}$) were diluted at 1:1 with normal saline fortified with 25%, 50% and 75% carrot juice, respectively, and stored at room temperature (27.0±0.2°C). Sperm motility and liveability reduced ($p<0.05$) as time of storage increased. in vivo study was carried out with 70 hens randomly allocated and inseminated with each of the 5 treatments. The fertility among the treatments was 75% (T_{NS}), 100% (T2), 60% (T3), and 83.33% (T4), while optimum of 60% at day 3 and 50% at day 7 were obtained for T3 and T5 respectively. Duration of fertile period was prolonged by carrot juice for $T_{50\%CJ}$ and $T_{75\%CJ}$ respectively by 2 and 3 days relative to T_{NS} and 5-6 days for T_{MRS}. Embryonic mortality and hatchability were similar among the treatments.

***Keywords:** Nera Black Breeder Cock, Carrot juice, Semen extension, Fertile period*

Description of Problem

Poultry play a significant role in producing and providing animal protein most effectively within the shortest possible time [1] due to their multiple egg production and faster growth rate. However. One of the problems limiting poultry production in Nigeria is low reproductive rate in terms of fertility due to unsuccessful mating, and hatchability [2]. It is no doubt that artificial insemination in poultry can be more beneficial when compared to natural mating and can be adopted to minimise reproductive problems in poultry [3, 4]. The increasing importance of artificial insemination in poultry reproduction has led to several researches on ideal condition for liquid (short-term) and frozen (long-term) semen storage. The possibility of dilution and storage of avian semen improve the work of poultry breeders and make it much easier by enabling them to conveniently transport semen to far away farms, to inseminate large group of hens, and to improve the utilisation of sperm from superior cocks [5].

The most common procedure for short-term avian semen storage (hours to days at refrigerator temperature) requires diluting sperm in an extender to retain their viability *in vitro*. Extenders serve to also protect the sperm cells from chemical and physical changes, contamination in their environment and provide more favourable conditions for fertilisation [6].

Diluents are buffered salt solutions used to extend semen to maintain the viability of

spermatozoa *in vitro*, and maximise the number of hens that can be inseminated. Semen diluents are based on the biochemical composition of chicken and turkey semen [7]. Addition of various components of extender to semen maintains sperm motility, fertilising capacity and preserve sperm membrane integrity [8, 9].

Semen is usually extended for preservative and protective purpose for future use. Hence, the need for semen extenders that can protect the sperm cells and prevent damage during storage. Good extender should provide energy for metabolic activities within sperm cell; maintain osmotic pressure and pH of the medium [10].

Carrot (*Daucus carota*) is a root fruit crop that contains carotenoids which are helpful in preventing damage of sperm cells from free radicals during storage due to its antioxidant properties, and it also have some significant health promoting properties. Several nutritional therapies have shown carrot to improve sperm counts and motility [11]. The consumption of carrot and its products is increasing steadily due to its recognition as an important source of natural antioxidants having anticancer activity [12, 13]. Carrots have long been said to be good for vision [14]. Recently, they have been proved to improve fertility in men and male animals [15].

Researchers investigating the effect of fruit and vegetables on the health of sperm discovered that carrots produced the best all-round results [16]. They had the greatest effect on 'motility' – a term used to describe the ability of spermatozoa to swim towards an egg [17]. Antioxidants help to neutralise free radicals, which are destructive groups of atoms and also by-product of metabolism. They are known to damage cell membranes and DNA [16]. Antioxidants can directly decrease the oxidative damage by reacting with free radicals or indirectly, by inhibiting the activity or expression of free radical generating enzymes or enhancing the activity or expression of intracellular antioxidant enzymes. Carrot fruit is a rich source of antioxidant which had been used to boost the quality and quantity of sperm cells *in vivo* when fed to the animal, however, there is limited information on the use of its juice as a medium for extending semen. Hence this study was designed to assess the effect of incorporating carrot juice in normal saline to extend cock semen on spermatozoa quality *in vivo* and *in vitro* at room temperature.

Materials and Methods

Experimental site

The research was carried out at the Poultry unit of the Teaching and Research Farm and Animal Physiology Laboratory, Department of Animal Science, University of Ibadan, Ibadan.

Management of experimental Animals

Ten cocks of about 52 weeks of age and Seventy hens were used for the experiment. The cocks and hens were reared on deep litter and battery cage system, respectively. The experimental animals were acclimatised for a period of three weeks before the commencement of the experiment. During this period, the cocks were trained for semen collection. Feed and water were provided *ad libitum* to the birds.

Processing of Carrot (Daucus carota) extract to be used as extender.

Fresh carrot fruits were obtained from the open local market in Bodija, Oyo state. The carrot was washed, scrapped to remove dirt and was put into a juice extractor so as to extract the carrot juice. The carrot juice was then centrifuged for 15minutes at 3000rpm to remove the sediment and the supernatant juice was decanted using a Pasteur pipette.

The extracted juice was stored at 4℃ for later use.

Preparation of the extenders

The extenders were prepared by incorporating carrot juice into normal saline (NS) at varying levels. Normal saline (0.9% NaCl solution) was bought from a pharmacy shop. Modified ringers' solution was prepared in the laboratory which contains 0.68g NaCl, 0.173g KCl, 0.064g $CaCl_2$, 0.025g $MgCl_2$, 0.025g $NaHCO_3$ composition dissolved in 100mls of distilled water as described by Kharayat (2016). The extender was then prepared as follows:

25% carrot extender = 25% carrot juice (CJ) +75% normal saline (NS)

50% carrot extender = 50% carrot juice (CJ) + 50%normal saline (NS)

75% carrot extender = 75% carrot juice (CJ) + 25% normal saline (NS)

Experimental Design and Layout

Experiment 1: In vitro study (Effect of carrot juice extender on semen quality)

Semen was collected from 6 cocks, pooled, and evaluated. Pooled semen was divided into 6 portions. Group T1 was left undiluted, while T2-T6 was extended at ratio 1:1.

T1- Undiluted semen
T2- Normal saline 100%(NS)
T3- Modified Ringer's solution 100%(MRS)
T4: 25% CJ +75%NS
T5: 50% CJ + 50%NS
T6: 75% CJ + 25%NS

Semen quality assessment was done using a microscope to compare the varying levels of CJ and NS in the extender to undiluted and MRS diluted semen which both served as controls. Treatment groups were evaluated *in vitro* for progressive sperm motility and livability under the microscope. The assessment was done hourly at room temperatures until motility drops below 50%, while the temperature of the extended and raw semen was maintained at 37^{o}C by keeping it in a regulated water bath

Experiment 2: In vivo study: Fertility response of layer breeder hens to carrot juice extended semen at different levels

Experimental animals

70 Nera black layer breeder hens were randomly allotted to 5 treatments in experiment 2 with 14 hens per treatment. Semen was collected from layer breeder cocks, pooled and extended and inseminated to the hens.

Artificial insemination Procedure

Hens in treatments 1-5 were inseminated with the various extended semen with the various extenders (100%NS, 100%MRS, 25%CJ, 50%CJ and 75%CJ). The insemination was done for 2 consecutive days initially at the first week and eggs were collected for a period of 4weeks. The procedure for the insemination is as follows: A sterile tuberculin syringe with an inseminating rod attached to it was used. The hens were everted to bring out the oviduct. 0.04ml of the extended semen from the various treatment groups was then deposited at a few inches into the vagina.

The insemination was carried out in the evening at about 5pm when it was confirmed that there were no more eggs in the uterus.

Egg collection and incubation

Artificial insemination day was recorded as day 0 of fertilisation in the hen. After a single insemination of two consecutive days, eggs were collected daily from the day after second day insemination. Eggs were collected daily, graded and stored at temperatures between 16 -18 ℃, prior to incubation. Cracked and dirty eggs were not

incubated and were discarded. On the 18th day of incubation, all eggs were candled, to identify the fertile eggs. Only fertile eggs were transferred from setter to hatcher in the hatchery for a period of 3 days. The fertility, number of hatched and dead chicks were counted and recorded. The eggs were collected for a period of 4 weeks.

Data collection

The cocks were trained for semen collection before the commencement of the experiment. After which semen was harvested twice a week and the ejaculate was taken to the laboratory for *in vitro* analysis. Semen was usually collected by the dorsal-abdominal massage method [18] and maximum care was ensured to avoid ejaculate being contaminated by faeces, blood or dirt during the collection.

The eggs were collected daily from each treatment and each egg was marked so as to indicate the treatment it belongs. Eggs that were collected from the treatments was stored and transported to the hatchery once a week. Percentage fertility, percentage hatchability, percentage embryo death, percentage hatch of setting, percentage hatch of fertile was calculated.

*Percentage fertility***:** percentage fertility was obtained by counting the number of fertile eggs divided by the total number of eggs set and multiply by 100.

$$\text{Fertility (\%)} = \frac{\text{No of fertile eggs}}{\text{No of egg set}} \times 100$$

Percentage hatchability**:** percentage hatchability was obtained by counting the number of chicks hatched from the hatching basket divided by the total number of fertile eggs and multiply by 100.

$$\text{Hatchability (\%)} = \frac{\text{No of hatch eggs}}{\text{No of fertile eggs}} \times 100$$

Embryo death**:** this was calculated by segmenting the embryo into the stages of development before death and it was classified into either early, mid or late embryonic mortality using the chick's development embryo chart. The early embryo death is between days 0-7 of development, mid embryo death is between days 8-17 while late is between days 19-20. The formula used was:

$$\text{\% Early embryo death} = \frac{\text{No of early embryo death}}{\text{No of fertile eggs}} \times 100$$

$$\text{\% Mid embryo death} = \frac{\text{No of mid embryo death}}{\text{No of fertile eggs}} \times 100$$

$$\text{\% Late embryo death} = \frac{\text{No of late embryo death}}{\text{No of fertile eggs}} \times 100$$

$$\text{\% Total embryonic death} = \frac{\text{No of early+ no of mid+ no of late}}{\text{No of fertile eggs}} \times 100$$

Percentage hatch of set eggs**:** percentage hatch of set egg was obtained by calculating the number of eggs hatched from the fertile eggs and then dividing it by the total number of eggs set multiply by 100

$$\text{\% Hatch of set eggs} = \frac{\text{No of eggs hatch}}{\text{No of eggs set}} \times 100$$

Data analysis

The data obtained were subjected to descriptive statistics and one-way analysis of variance using statistical analysis software version 9.3 [19]. The means were compared using Duncan multiple range test of the same software.

Results

Characteristics of pooled raw semen collected

The characteristic of pooled raw semen used in this study is presented in Table 1: The sperm motility, sperm concentration, Livability and colour were 87.5%, 1.05×10^9,

92.5% and creamy white respectively. Which indicated that the semen was of good quality.

Table 1: Characteristics of pooled raw semen

Parameters	Values
Progressive sperm motility (%)	87.5±3.54
Semen Colour	creamy white
Mass activity	+++
Semen volume (mL)	0.5±0.2
Sperm Concentration (×10^9)	1.09±0.67
Sperm cell Liveability (%)	92.5±3.54

Sperm motility (%) of the cock semen extended with varying levels of carrot juice at room temperature (Temp-27.1 ^{O}C, RH-76%)

The result of sperm motility of un-extended and extended semen with varying levels of carrot juice and other diluents over time at room temperature (27.1^{O}C) is presented in Table 2. Results indicated that sperm motility dropped over time as storage time increases across the treatments, although there was no significant difference (P>0.05) among the treatments at each storage time. Values ranged from 73.33% (75%CJ) to 90.67% (US) for all the groups at the zero hour, while at the third hour of storage, sperm motility had dropped to 51.67%(US) and below 40% in other treatment groups.

Table 2: Sperm motility (%) of un-extended and semen extended with varying levels of carrot juice over time at room temperatures.

	Storage time			
Treatment	**0hr**	**1hr**	**2hr**	**3hr**
T1 (T_{US})	90.67±2.96	80.00±5.77	68.33±8.17	51.67±4.41
T2 (T_{NS})	82.67±1.45	74.67±2.60	48.33±1.67	31.67±10.92
T3 (T_{MRS})	77.33±2.33	69.33±2.33	50.00±5.77	31.67±7.26
T4 ($T_{25\%CJ}$)	87.67±3.93	71.33±11.41	43.33±12.01	31.00±11.15
T5 ($T_{50\%CJ}$)	80.33±10.17	68.33±19.22	37.67±13.86	35.33±12.67
T6 ($T_{75\%CJ}$)	73.33±16.83	66.33±18.17	39.33±19.68	31.33±15.71

NS (Normal saline), MRS (Modified ringer's solution), CJ (Carrot juice), US (Un-extended semen)

Sperm liveability (%) of the cock semen extended with juice (Temp-27.1 ^{O}C, RH-76%)

The result of the liveability of cock semen extended with varying levels of carrot juice and other diluents is presented in Table 3. Results indicated that sperm liveability drop over time as storage time increases across the treatments although there was no significant difference (P>0.05) among the treatments at each of the storage period. Sperm liveability for all groups was above 90% at the zero and first hour. At the second hour, it ranged from 62.3% (75%CJ) to 89.00% (NS) while at the third hour, sperm liveability of US (80%) and NS (83%) were significantly (P<0.05) higher than MRS (60%) and all the carrot juice-based extenders which had values of about 61%.

Table 3: Sperm liveability (%) of Layer breeder cock semen extended with varying levels of carrot juice over time at room temperatures.

	Storage time			
Treatment	**0hr**	**1hr**	**2hr**	**3hr**
T1(T_{US})	96.00 ^{O}C 2.08	92.67± 4.33	80.67± 12.99^{b}	80.00± 12.5^{a}
T2(T_{NS})	94.33± 4.70	90.33± 5.36	89.00± 0.58^{a}	83.00±11.68^{a}
T3(T_{MRS})	95.67± 1.76	84.33± 9.28	78.67± 9.40^{b}	60.00± 1.00^{b}
T4($T_{25\%CJ}$)	94.33± 2.19	81.67± 10.93	78.33± 14.24^{b}	61.33± 10.93^{b}
T5($T_{50\%CJ}$)	95.67± 2.96	87.33± 8.97	81.33± 10.72^{b}	61.00± 1.20^{b}
T6($T_{75\%CJ}$)	96.33± 3.18	89.33± 7.45	62.33± 31.20^{c}	61.60± 30.87^{b}

NS (Normal saline), MRS (Modified ringer's solution), CJ (Carrot juice), US (Un-extended semen)

Fertility trend of extended semen inseminated once for a period of 28 days

The percentage fertility trends of the eggs laid by the hens artificially inseminated once in two successive days is shown in Figure 1. The maximum fertility across the treatments occurs at day 4 with exception of T3 and T5. The fertility among the treatments were 75% (T1), 100% (T2), 60% (T3), and 83.33% (T4), while optimum of 60% at day 3 and 50% at day 7 were obtained for T3 and T5, respectively. The duration of fertile period of fertile period was prolonged by carrot juice for 50% (T4) and 75% (T5) respectively by 2 and 3 days relative to T1 and 5-6 days for T2.

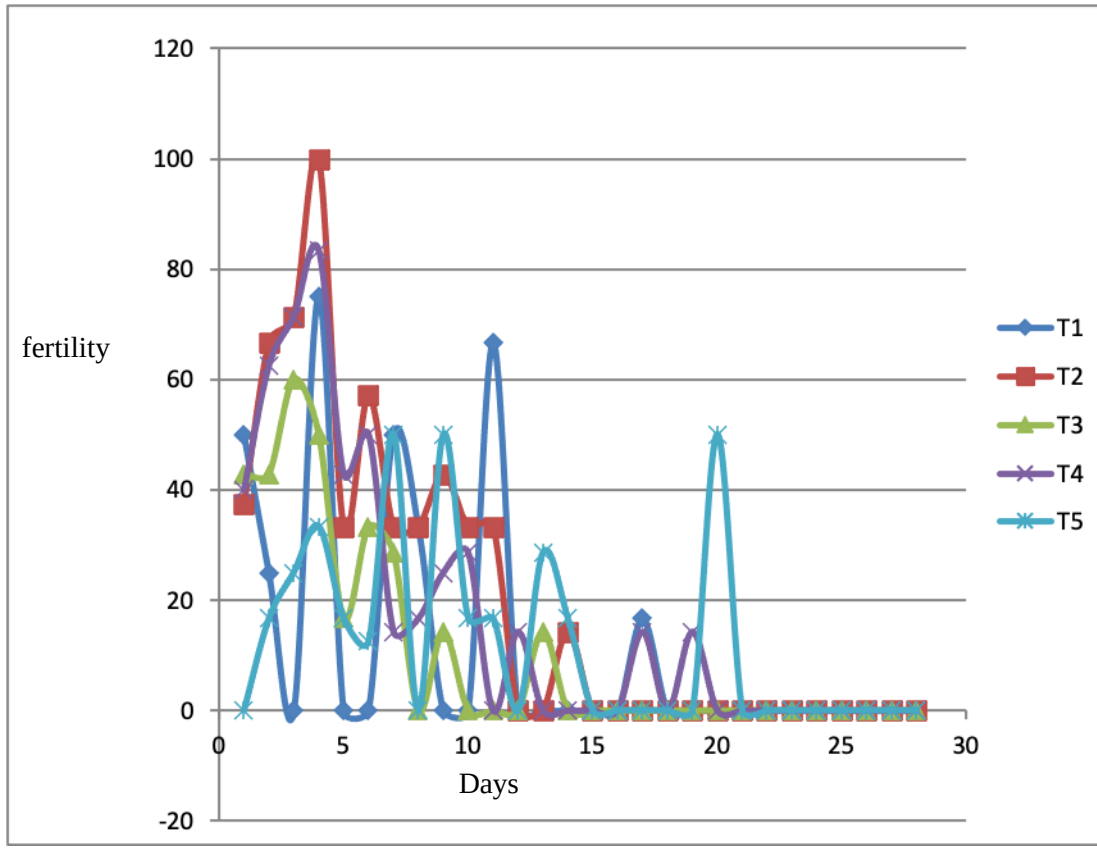

Figure 1: Fertility trend in days for all the treatments

Percentage weekly egg fertility post insemination

The percentage weekly fertility results are presented in Table 4. Fertility was significantly different across all the treatments at the first three weeks of the experiment but similar among treatments at week 4. At week 3, T1 recorded highest fertility (30.42%), while T2 and T3 had zero fertility.

Table 4: Egg fertility (%) from hen inseminated in 2 successive days only

Treatments	Week 1	Week 2	Week 3	Week 4
1(NS)	30.2±10.67^{ab}	5.55±5.55^{c}	30.42±12.48^{a}	0.00±0.00
2(MRS)	47.35±3.77^{a}	20.30±10.04^{a}	0.00±0.00^{b}	0.00±0.00
3(25%CJ)	38.68±5.45^{ab}	4.44±2.72^{c}	0.00±0.00^{b}	0.00±0.00
4(50%CJ)	38.55±14.59^{ab}	10.59±4.09^{b}	1.56±1.56^{b}	0.00±0.00
5(75%CJ)	13.81±7.98^{b}	19.42±10.9^{a}	4.17±4.17^{b}	0.00±0.00

a,b; means along the same column with different superscript are significantly (P<0.05) different
SEM: Standard error of mean, NS: Normal saline, MRS: Modified ringer's solution, CJ: Carrot juice

Percentage embryo mortality and egg hatchability

The percentage embryonic mortality (early, mid and late) and hatchability post insemination are presented in Tables 5, 6, 7 and 8. There were no significant difference (P>0.05) among all the parameters. The hatchability for T2 (80.71%) was the highest followed by T4 (71.43%) and T5 (50%) at week 1 while the total embryonic mortality was high for T1, T3 and T5 (88.88%, 77.08% and 50% respectively), while it was reduced in T4 and T2(28.57% and 19.29% respectively) for week 1.

Table 5: Early embryonic mortality across the treatments for a period of 4 weeks

Weeks	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5
1	55.55±22.22	5.00±5.00	35.42±14.58	14.29±8.25	25.00±14.43
2	12.50±12.50	46.25±21.73	25.00±25.00	0.00±0.00	75.00±25.00
3	25.00±25.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Trt 1(NS), Trt 2 (MRS), Trt 3 (25% CJ), Trt 4 (50% CJ), Trt 5 (75% CJ)

Table 6: Mid embryonic mortality across the treatments for a period of 4 weeks

Weeks	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5
1	25.00±15.96	14.29±10.10	16.67±11.79	10.71±10.71	0.00±0.00
2	0.00±0.00	0.00±0.00	0.00±0.00	50.00±28.87	0.00±0.00
3	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Superscripts along the same row and column indicates no significant difference (P>0.05)
Trt 1(NS), Trt 2 (MRS), Trt 3 (25% CJ), Trt 4 (50% CJ), Trt 5 (75% CJ)

Table 7: Late embryonic mortality across the treatments for a period of 4 weeks

weeks	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5
1	0.00±0.00	0.00±0.00	33.33±19.25	0.00±0.00	0.00±0.00
2	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Superscripts along the same row and column indicates no significant difference (P>0.05)
Trt 1(NS), Trt 2 (MRS), Trt 3 (25% CJ), Trt 4 (50% CJ), Trt 5 (75% CJ)

Table 8: Percentage hatchability (Hatch of fertile) on a weekly basis

Weeks	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5
1	11.11±11.11	80.71±8.91	22.92±15.73	71.43±16.49	50.00±0.00
2	12.50±12.50	28.75±18.07	25.00±25.00	25.00±25.00	0.00±0.00
3	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Superscripts along the same row and column indicates no significant difference (P>0.05)
Trt 1 (NS), Trt 2 (MRS), Trt 3 (25% CJ), Trt 4 (50% CJ), Trt 5 (75% CJ)

Discussion

Semen characteristics are important indicators of the reproductive potentials of breeding cocks. The characteristics of the pooled raw semen evaluated in this study was observed to be adequate according to Mkpughe and Bratte [20]. The creamy white colour of the semen is good quality semen with a high concentration of sperm cells. This finding agrees with the previous report that good cock semen should have a creamy whitish colour [20]. Ejaculate volume per cock (0.2±0.1ml) observed in this study was a little similar to an average of 0.28mL reported by Peters *et al.* [21] per cock in which differences may be due to location, climate, nutrition, management, and possibly semen collection procedures. Sperm motility (87.5±3.54%) of the pooled semen in this study was higher than the range of motility from fresh cock semen (73.9 to 83.2%) as earlier reported [22, 23]. The concentration of sperm cells contained in the raw semen used for this study was $1.09\pm0.67x10^9$/ml which is below the range of 3 to 7 $x10^9$/ml reported by Hafez and Hafez [24] for domestic cock. The difference in sperm concentration among the different breeds and lines of cocks may be attributed to several factors such as genetic variation, individual performance, generation, and stimulation during collection of semen.

When semen is stored 24 h or longer *in vitro*, fertility problems are magnified [25]. The addition of antioxidants to semen during preservation is done to protect the sperm cells against damage caused by ROS to motility, viability, energy production and DNA integrity [26, 27, 28]. The progressive motility in all the treatments at 0hr was not significantly different from one another. However, the groups with 25%CJ and undiluted semen had motility that was apparently higher than other treatment groups. This may be attributed to the ability of the juice to serve as an effective antioxidant and energy sources for the sperm cells to retain vigorous movement. This supports the report of Ball *et al*. [29] and Reza *et al.* [30] whose work revealed that sperm motility was significantly higher in treatments extended with vitamins E and C compared with the control group and other treatments. A close observation at the percentage live sperm cell under the microscope indicated that more than 94% of sperm cells were alive in all the treatments even with the addition of the conventional MRS and NS at 0hr. This indicated that the sperm liveability was not affected by these diluents.

At 1hr and 2hrs of storage under room temperature of 27.2°C and RH of 74%, there was a decrease in motility across all the treatments. This agrees with the earlier finding that sperm motility decreases with increased time of storage [31]. The unextended semen has the apparently highest sperm motility across the treatments. This could be compared to the findings of Blesbois *et al.* [32] that observed the proportion of motile spermatozoa was high in fresh semen (87%) and significantly lower

(46%) in semen stored at 4°C for 48 hours. While there was a rapid decline in the proportion of live sperm cells at the second hour which agrees with the findings that during the time of storage, there is a decrease in live, morphologically normal spermatozoa and an increase in dead spermatozoa and spermatozoa with bent necks [32, 33].

The decrease in motility across the treatment containing diluents may be due to a reduce pH of the diluents or extender [25]. The observed drop in motility across all the treatments below 40% except for the unextended semen (T1) which was still above 50% at 3hr agree with Elagib *et al.* [34] that observed a decline in motility when semen samples was held at 30°C for 2hrs and 4hrs. The carrot extended semen treatments still maintained a relatively higher numbers of live spermatozoa (61.33%, 61.00% and 61.60%) respectively at the third hour which may implies that the antioxidant component of carrot was still able to keep sperm cells alive up to 3hrs [35] but might not be very potent to still supply energy for effective motility of the sperm cells. This supports the findings of Elagib *et al*. [34] that observed that the preservation of motility of sperm cells during storage was reduced when no energy substrate was present in the extender. High temperatures result in a higher metabolic rate and such causes faster depletion of all critical nutrients, hence a rapid decline in motility.

In this study increasing the storage time of the cock semen to 3hr resulted in the increase % of dead spermatozoa similar to the results observed by Clark *et al.* [36]. They reported that the percentage of dead sperm cells at 5°C and 10°C were lower than at 30°C. This could be attributed to a rise in metabolic activity and depletion of substrate materials that resulted in increased metabolic by products concentration and accumulation of lactic acid which reduce the pH with ultimate sperm cell death [36].

Fertility duration is the overall number of days which a hen lays fertile eggs after a single artificial insemination [37, 38] and is determined largely by the amount of sperm cells stored in the sperm storage tubules of the hen [7]. Maximum fertility for the individual treatment varies due to the fact that the semen was extended with different diluents for each treatment which forms the basis of the comparison. The maximum fertility across the treatments observed at day 4 in this study for semen extended with 50%CJ was comparable with conventional modified ringer solution and this is similar to the reports of Tabatabei *et al.* [39] that maximum fertility was at day 3 which was constant till day 5. Although fertility trend across all the treatments daily was not constant which might be due to age of the birds as reported by King'Ori [40] and Ewuola et al. [41].

The duration of fertile period observed in this study was prolonged by carrot juice at 50% and 75% concentration by 2 and 3 days, respectively relative to normal saline diluents 5 to 6 days, respectively relative to the conventional extender. This could be attributed to antioxidants in the carrot which possibly boosted the viability of the extended spermatozoa and their longevity in the SST. The duration of fertile period in this experiment agrees with the report of Tabatabei *et al.* [39] who also observed that the duration of fertility was obtained at day 19. The fertility across all the treatments was below 50% post insemination at day 1 except for normal saline extended semen which has 50% fertility on this day. This corroborates the findings of Bobr *et al.* [42] that low fertility was observed within 24hours following insemination conducted right after oviposition. Also, the reduced fertility can be attributed to the capacity of the sperm

storage tubules (SST) in the hens to hold spermatozoa for a very long time. According to the reports of Bakst *et al.* [43] and Adebisi and Ewuola [38] that the biological basis of sustained fertility in chicken and turkey hens is in their capacity for sperm to reside in the SSTs of the utero vaginal junction.

Fertility at the first week post insemination was not significantly different among the treatments except for treatments with modified ringer's solution and 75% carrot juice which were significantly different from each other. Modified ringer's solution having the highest fertility of 47.35% and 75% carrot juice having a fertility percentage of 13.81%. This may be as a result of the concentration of the extender in 75% carrot juice being more than the normal medium at which sperm cells can effectively swim *in vivo* in the female genital tract thereby causing a very low fertility in that treatment.

At week 2, the fertility of all the treatments that reduced drastically though without significant difference across all the treatments could be attributed to the fact that SST is gradually becoming depleted of sperm cells since there was no repeated insemination which may be required to fill up the SST. At weeks 3 and 4, fertility across all the treatments was tending towards zero except for normal saline, 50% carrot juice and 75% carrot juice treatments which still have a fertility of 30.42%, 1.56% and 4.17%, respectively, while others were zero at week 3. All the treatments were already zero at week 4 which can be attributed to the fact that the population of sperm cells in the SST were not sufficient to cause fertility or probably the available sperm cells were either dead or stale. These results were in agreement with Saleh dan Sugyatno [44] and Obidi *et al.* [45] that sperm numbers may affect the *in vivo* storage of spermatozoa and subsequently the fertile period [38].

Total embryonic death for normal saline, 25% carrot juice, and 75% carrot juice (88.88%, 77.08% and 50%) were significantly higher than 50% carrot juice and modified ringer's solution (28.57% and 19.29%) which might be as a result of the age of the hens. Tabatabei [39] reported that some of the causes of embryonic mortality are age of the breeder bird, season of the year, egg storage, egg size among others. This can also be as a result of increasing oviductal sperm storage resulting in old and stale sperm cells in the oviduct leading to embryonic death [41]. The early embryonic death across the treatments for the period of 4 weeks were all below 50% except for normal saline with 55.55% in week 1 and 75% carrot juice in week 2 (75%) which may be attributed to a poor storage of the eggs during incubation due to the early embryonic mortality occurring during or after the 1st week of incubation [39].

Hatch of fertile was similar among the treatments but normal saline (80.71%) and 50% carrot juice (71.43%) had a higher percentage of hatch of fertile at week 1. However, the poor result in the hatch of fertile across all the treatments might be attributed to environmental condition of the incubator as described by King' Ori [40] that mortality is seen in eggs in the incubator if the temperature drops below 35.6℃ or rises above 39.4℃ for a number of hours.

Conclusion and Application

The result from this experiment suggests

1. That carrot juice did not improve spermatozoa quality *in vitro* in the extended semen when stored at room temperature which might be as a result of the duration of storage. Although further research should be carried out on the assessment of the pH of the extender to verify if it could be a major factor that needs to

be buffered if the carrot juice will be used for further studies in extending cock semen.

2. The result of the *in vivo* experiment revealed that carrot juice at 50% and 75% concentration for extending cock semen is able to prolong the duration of fertile period in inseminated hens by 2 and 3 days compared to normal saline and 5 to 6 days compared to the modified ringer solution conventional extender
3. Therefore, Carrot juice can be recommended to be used as a source of extender for on-farm artificial insemination**.**

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