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Effects of semen dosage, oviductal sperm storage and insemination interval on egg fertility, embryo mortality and hatchability in Nera Black breeder chickens

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

Animal Physiology and Bioclimatology unit,
Department of Animal Science, University of Ibadan, Ibadan, Nigeria
***Corresponding author:** eoewuola@gmail.com; +2348060862361

Abstract

The biological basis of sustaining fertility in poultry is their ability to store sperm cells in the sperm storage tubules (SST) located in the uterovaginal junction. However, artificial insemination in poultry industry is haphazardly administered in Nigeria without regulation on semen dose and frequency of insemination for optimum fertility. The objective of this study was to establish a semen dosage and insemination interval for maximum fertility and embryonic survival in Nera black layer breeder chickens. A total of 80 breeder hens (52 weeks) were allotted to five (5) treatments with four (4) replicate per treatment. Semen was pooled from 10 matured breeder cocks and inseminated to four groups of hens at varied semen dose of 0.02mL (T1), 0.04mL (T2), 0.06mL (T3) and 0.08mL (T4) of undiluted semen while hens in T5 were mated naturally, both for two consecutive days. 0.02, 0.04, 0.06 and 0.08mL of pooled semen contained 20.43×10^6, 40.87×10^6, 61.30×10^6 and 81.74×10^6 motile spermatozoa. Eggs were collected, stored and artificially incubated weekly for 4 weeks. Fertility, embryo mortality and hatchability parameters were determined. Another 78 breeder hens were allocated into 4 treatments of 5 replicates per treatment with unequal number of hens and were inseminated with 0.02mL of raw semen containing 20.43×10^6 motile sperm cells at 3, 6, 9 and 12 days intervals. Fertility, hatchability and embryo mortality were determined. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05.}$ Hatch of fertile eggs in T5 at week 2 (65.36±13.28) was significantly higher ($p<0.05$) than T1 (33.83±12.65), T2 (13.25±6.88), T3 (39.17±14.17) and T4 (28.21±11.37). At weeks 1 and 2, there was no significant different across the treatments. Fertility at 4 weeks in T1 (11.53±6.66) was significantly ($p<0.05$) different from treatments T2 (0.00±0.00), T3 (0.00±0.00), T4 (1.66±1.66) and T5 (0.00±0.00). Total and early embryo mortality in week 3 was significantly higher ($p<0.05$) in T1 (100.00±0.00, 95.00±5.00) than in treatments T2 (43.75±0.00, 43.75±25.77), T3 (66.67±23.57, 66.67±23.57), T4 (95.00±5.00, 85.00±15.00) and T5 (37.50±23.94, 22.92±15.73). Fertility was significantly ($p<0.05$) higher in 3 days insemination interval (52.65±7.25) compared with 6 days (39.87±4.70), 9 days (22.98±5.71) and 12 days (36.14±6.89). At weeks 1 and 3, the hatch of fertile eggs across the treatments was not significantly ($p>0.05$) different from one another. This study suggests that inseminating semen dose of 0.02mL containing approximately 20.43×10^6 motile sperm cells in Nera black layer breeder chickens will give a maximum fertile period of 5 days, while insemination interval of 3 days using 0.02mL of semen gave highest fertility level.

Keywords: Sperm Storage Tubules, Fertile period, Insemination interval, Layer breeder chickens.

Introduction

In poultry production, fertility needs to be improved upon so as to get more chicks and invariably table eggs for production. Availabilty of day-old chicks is a pre-requisite for any anticipated expansion in the poultry industry. In order to increase fertility in poultry, farmers tend to adopt artificial insemination ahead of natural mating. It has been established that artificial

insemination in avian species has relative advantages compared with natural mating (Penfold *et al.,* 2000; Brillard, 2003). These advantages include increased number of settable eggs, better overall fertility and hatchability, thus reducing the cost of production per unit of day-old chicks (Brillard, 2003). Several factors need to be considered for optimum success of artificial insemination and they include breeder stock management, semen quality and quantity, semen dosage, depth of insemination, frequency and timing of artificial insemination (King *et al.,* 2002). A cock can fertilize 6-10 hens in a flock under natural mating. But in case of artificial insemination, semen collected from 6-8 males can be used to inseminate 150-170 females (Habibullah *et al.,* 2015).

Fertility in chicken under artificial insemination can be affected by series of factors such as insemination interval, number of spermatozoa inseminated, semen quality, semen dosage and timing of insemination. Different dosage of semen had been reported to give different optimum fertility. Saleh *et al.* (2012) have demonstrated that insemination with 50 million sperm cells would be adequate for achieving optimum fertility and fertile period. In poultry, the utero-vaginal junction (UVJ) of the oviduct contains SSTs, where the most fecund sperm cells are stored (Bakst, 1998).

Sperm storage sites in the UVJ have been referred to by several names such as vaginal glands, sperm glands, UV sperm-host glands, sperm storage tubules and UV sperm-storage glands (Ito *et al*., 2011; Sasanami *et al*., 2013; Khillare *et al*., 2018). Prolonged storage of sperm by female birds after mating is known to occur in several species, with mean sperm storage durations ranging from about **6** days to 45 days (Birkhead, 1988). Only 1% of the deposited sperm passes through the selection process in the vagina to reach the UVJ (Khillare *et al.,* 2018). The duration of sperm storage in the SSTs is species-dependent. Chickens can store sperm for up to three weeks, whereas turkeys can maintain sperm for 10 weeks in the SST and still lay a fertilized ovum (Christensen and Bagley, 1989 and Brillard *et al*., 1998).

The adaptive significance of prolonged sperm storage in birds is not well understood. However, this study was designed to assess the effect of semen dosage, oviductal sperm storage and insemination interval on fertility, hatchability and embryo mortality in Nera black layer breeder hens.

Materials and methods

Experiment 1

Effects of undiluted semen dosage and oviductal sperm storage on fertility and embryo mortality in Nera Black layer breeder chickens

Experimental location

The study was conducted at the Poultry Unit of Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. Ibadan is located 100 miles away from the Atlantic coast in the South west region of Nigeria. It is a rain forest zone about 200m above sea level.

Experimental animals and semen collection

Eighty breeder hens and ten matured breeder cocks were used for this experiment with an average age of 52 weeks. The hens were randomly allocated to five (5) treatments with four (4) replicates per treatment. The experimental hens were housed in 3-tier battery cages, while the cocks were reared on deep litter system. Both hens and cocks were fed 1110 – 120g of feed/day and clean fresh water was made available to the animals throughout the period of the experiment. The semen was collected by dorsa-abdominal massaging of the cocks as described by Burrow and Quinn (1937) into a collection tube.

Artificial insemination

Abdominal massage to evert the genital tract of the breeder hens was done according to the method of Lake and Stewart (1978). Thereafter, the graduated tuberculin syringe with glass rod that contained pooled semen was inserted into the hen's vagina and the semen was released intravaginally, before the vagina started to relax. The hens were inseminated with different doses of semen (0.02, 0.04, 0.06, and 0.08mL) for two consecutive days across all treatments the semen collection inseminations were done in the evening (when the ambient temperature was lower and also to minimize accumulation of the hard shell eggs in the uteri of the hens).

Semen evaluation

The semen collected from the cocks were pooled together and measured with the aid of graduated tuberculin syringe. Semen colour was assessed visually to ensure it was devoid of blood stain, dirt and any other contaminants before further process for A.I. Any abnormal semen samples noticed was generally discarded. Sperm mass activity was determined with the aid of binocular microscope at a magnification of x100 according to Ewuola and Egbunike (2010). Mass activity was scored on a scale of "+" to "++++" according to the intensity to wave generated by the sperm cells. Sperm motility was evaluated according to Ewuola and Egbunike (2010) and also with binocular microscope at magnification of x400. Progressive motile spermatozoa were rated between 0 to 100%.

Sperm concentration was evaluated with the use of a New Improved Neubauer Haemocytometer and a binocular microscope as outlined in Ewuola and Egbunike (2010). The ratio of live sperm cells to dead sperm cells was carried out by staining a drop of raw semen with eosin-negrosin, smeared and viewed under the microscope after air-dried for few seconds. Dead sperm cells absorbed the stain while live sperm cells did not. Sperm concentration was determined using this formula:

C = n x d x 50000

Where C = sperm concentration

n = number of sperm cells counted in 5 diagonal squares

d = dilution factor

50000 is constant

Egg collection and incubation

Egg collection begun about 48 hours after the first insemination. Eggs were properly marked, stored at room temperature and set at 7 days interval for 4 weeks.

Determination of the duration of fertile period

The duration of sperm storage is defined here as the length of time for which females can lay fertile eggs after their last insemination (Birkhead and Moller, 1992). Eggs were collected, marked, set in the hatchery weekly and incubated till fertility got to zero after artificial insemination.

Experiment 2

Effects of insemination interval on fertility and embryonic mortality in Nera Black layer breeder chickens

Two weeks after the first experiment, the hens were re-randomised and allocated into 4 treatments of 5 replicates with unequal number of hens in each treatment in a completely randomised design. The dose for insemination for this experiment was 0.02mL of undiluted semen containing 20.43×10^6 number of motile sperm cells. The semen ejaculate from the cocks was pooled together, measured and a portion of the semen was evaluated for progressive sperm motility, sperm liveability and sperm concentration as described by Ewuola and Egbunike (2010). Insemination was done at different intervals; treatment 1 (T1) at 3 days, treatment 2 (T2) at 6 days, treatment 3 (T3) at 9 days, treatment 4 (T4) at 12 days interval.

Determination of fertility and embryo mortality

The fertility was determined by candling eggs on day 18. Eggs classified as infertile were broken up and examined to check for embryonic mortality and the number of infertile eggs per treatment were noted and classified as described by Brillard *et al.* (1998). Those eggs with dead embryo were added to the fertile eggs. Percentage fertility was estimated by dividing the number of fertile eggs by total number of eggs set multiplied by 100

Statistical analysis

All data obtained were subjected to descriptive statistics and one-way analysis of variance (ANOVA) using statistical analysis software (SAS) version 9.3 (SAS, 2011). The means were compared using New Duncan's Multiple range test.

Results

The mean values of pooled semen inseminated on the two successive days of insemination is presented in Table 1. The sperm motility ranged from 80 to 85% with mean sperm concentration of 1.20×10^9.

Effect of semen insemination dose on fertility, embryo mortality and hatch parameters in layer breeder chickens after two successive days of insemination

The result of semen insemination dose on fertility in Nera black layer breeder is presented in Table 2. Percentage fertility in the first week ranges between 61.20±4.85% (T3) to 62.11±13.15% (T2) and was significantly ($p>0.05$) higher than T1 (58.01±10.62%). At 2 and 3 weeks post insemination, there was no significant difference among the treatments in percentage fertility. At 4 weeks post-insemination, egg fertility in T1 was significantly ($p<0.05$) higher than T2, T3, T4 and T5, while egg fertilty in T2, T3, T4 and T5 was statistically similar ($p>0.05$). T2, T3 and T5 were infertile. At the 4th week of post-insemination, eggs in T2. T3 and T5 were infertile while T4 (1.66±1.66) was significantly low.

At week 1, late embryo mortality in T4 was significantly ($p<0.05$) higher than other treatments (T2, T3, T5) but similar to T1. There was no significant difference across treatments in hatch of fertile, hatch of egg set, early, mid and total embryo mortality at first week post insemination.

At the week 2, hatch of fertile T5 (65.36±13.28%) was significantly ($p<0.05$) higher than T2 (13.25±6.88%) and T4 (28.21±11.37%) but similar to T1 (33.83±12.65%) and T3 (39.17±14.17%). There was no significant difference across the treatments for total embryo mortality.

At week 3, the early embryo mortality in T1 (95.00±5.00%) and T4 (85.00±15.00%) were similar but these were significantly ($p<0.05$) higher than 43.75±25.77%, 66.67±23.57% and 22.92±15.73% obtained for eggs set in T2, T3 and T5. The total embryo mortality of egg set in T2 (43.75±0.00%) and T5 (37.50±23.94%) was significantly lower ($p<0.05$) than T1 (100.00±0.00%) but statistically similar to T3 (66.67±23.57%) and T4 (95.00±5.00%). The total embryo mortality reached 100% at week 3. At week 4, early, mid, late and total embryo mortality were not significantly different across the treatments. Hatch of fertile, hatch of set with mid and late embryo mortality were all zero at 4th weeks of post insemination.

Effect of oviductal spermatozoa age and duration of fertile period on fertility in Nera black layer breeder chickens

The duration of the fertile period and effect of oviductal spermatozoa age on fertility in layer breeder chicken is shown in Figure 1. This showed the mean value of fertility for 28 days in breeder layer chickens inseminated for two successive days. The maximum fertile period of 81.67%, and 81.05% was observed at days 3 and 4 post insemination but there was no significant difference between the days. Lowest

fertility of 0% at days 26 and 27, and 5.56% at day 28 were observed with significant difference ($p<0.05$). Fertility response to oviductal age at days 1, 2, 3, 4 and 5 were 59.58%, 70.35%, 81.67%, 58.30% and 81.05%, respectively.

Table 1: Semen characteristics of pool semen inseminated on two consecutive days

Semen Parameters	Mean Values ± Standard Deviation	Range
Spermatozoa mass activity	++++	++++
Progressive spermatozoa motility (%)	82.50±2.50	80 - 85
Spermatozoa Concentration (×10^9/ml)	1.20±0.22	0.98 - 1.42
Spermatozoa Liveability (%)	98.50±0.01	97-100

Table 2: Effect of Semen Insemination Dose on egg fertility in Layer Breeder Chickens after Two Successive Days of Insemination (Mean±S.E)

Weeks	Treatments	Number of Egg Set	Fertility (%)	Embryo Mortality (%)				Hatch of Fertile (%)	Hatch of Egg Set (%)
				Early	Mid	Late	Total		
1	T1	70	58.01±10.62	39.26±16.68	6.67±6.67	1.67±1.67^{ab}	47.60± 12.91	38.56±16.00	25.57±11.77
	T2	62	62.11±13.15	42.26±11.25	0.00±0.00	0.00±0.00^{a}	46.26±11.25	47.02±8.69	29.27±9.14
	T3	70	61.20±4.85	10.27±5.90	25.00±25.00	0.00±0.00^{a}	35.27±22.10	55.18±19.98	36.44±14.19
	T4	58	64.35±6.43	35.17±9.19	9.38±9.38	7.67±4.58^{b}	52.22±9.34	41.53±8.06	27.62±7.24
	T5	47	62.11±16.42	22.78±11.90	6.97±4.07	0.00±0.00^{a}	29.75±11.72	70.25±11.72	40.56±11.56
2	T1	61	42.05±5.27	63.39±12.10	0.00±0.00	2.78±2.78	66.17±12.65	33.83±12.65^{ab}	14.07±5.27
	T2	52	45.65±16.13	62.82±18.89	2.56±2.56	10.26±10.26	56.73±21.49	13.25±6.88^{a}	5.67±3.60
	T3	63	37.78±11.57	21.46±9.24	28.13±24.14	11.25±6.57	60.83±14.17	39.17±14.17^{ab}	19.44±8.77
	T4	55	52.93±11.01	66.79±11.27	5.00±5.00	0.00±0.00	71.79±11.37	28.21±11.37^{a}	16.96±9.28
	T5	41	41.33±8.04	23.39±8.43	11.25±6.57	0.00±0.00	34.64±13.28	65.36±13.28^{b}	27.20±7.49
3	T1	50	25.16±6.21	95.00±5.00^{b}	0.00±0.00	5.00±5.00	100.00±0.00^{b}	0.00±0.00	0.00±0.00
	T2	54	9.34±3.56	43.75±25.77^{ab}	0.00±0.00	0.00±0.00	43.75±0.00^{a}	31.25±23.66	3.59±2.37
	T3	58	11.44±5.30	66.67±23.57^{ab}	0.00±0.00	0.00±0.00	66.67±23.57^{ab}	8.33±8.33	2.08±2.08
	T4	61	14.61±5.77	85.00±15.00^{b}	5.00±5.00	5.00±5.00	95.00±5.00^{ab}	5.00±5.00	1.56±1.56
	T5	41	11.25±6.57	22.92±15.73^{a}	14.58±8.59	0.00±0.00	37.50±23.94^{a}	12.50±12.50	3.13±3.13
4	T1	50	11.53±6.66^{b}	50.00±28.87	0.00±0.00	0.00±0.00	50.00±28.87	0.00±0.00	0.00±0.00
	T2	51	0.00±0.00^{a}	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	T3	50	0.00±0.00^{a}	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	T4	41	1.66±1.66^{a}	25.00±25.00	0.00±0.00	0.00±0.00	25.00±25.00	0.00±0.00	0.00±0.00
	T5	40	0.00±0.00^{a}	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

$^{a, b}$: Means on the same column with different superscripts differ significantly (P<0.05). S .E = Standard error

Experiment 2: Effect of insemination interval on fertility and embryo mortality in Nera black layer breeder chickens

The characteristics of pooled semen inseminated for 5 weeks

The mean characteristics of pooled semen inseminated for period of 5 weeks is presented in Table 3. The average spermatozoa motality was 84.64±1.38% while the sperm concentration ranged from 1.42 to 2.10×10^9.

Effect of insemination interval on fertility, hatchability and embryo mortality from layer breeder chickens using 0.02mL of undiluted semen for 5 weeks

The mean values of number of egg set, fertility, hatchability, embryo mortality and hatch of set eggs from breeder layers of chicken using 0.02ml of undiluted at various interval for 5 weeks is presented in Table 4. The fertility in T1 (52.65±7.25) was significantly higher than T2 (39.87±4.70), T3 (22.98±5.71) and T4

(36.14±6.89). There was significant (P<0.05) difference across the treatments for egg fertility. Egg fertility in hens on T1 was statistically similar to T2 and T4 but significantly higher (p<0.05) than T3. There was no significant difference (p>0.05) across treatments for hatchability (%). The result shown for hatch of set in T1 was similar to T2 and T4 but significantly (p<0.05) higher than T3. The mid stage embryonic mortality in T3 was similar to T1 and T4 but significantly (p<0.05) higher than T2. There was no significant difference across treatments in early and late embryo mortality.

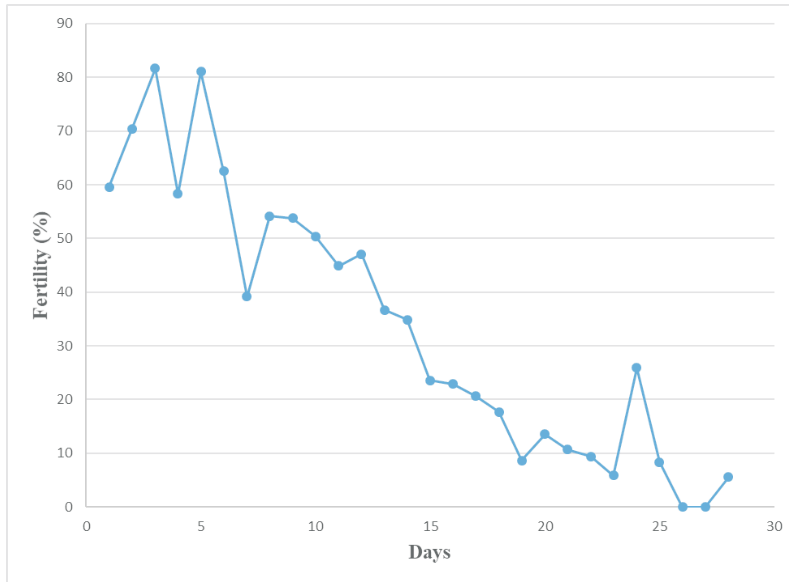

Figure 1: Effect of oviductal spermatozoa age and duration of fertile period on egg fertility in layer breeder chickens

Table 3: Semen characteristics of pool semen inseminated during insemination intervals

Semen Parameters	Mean Values ± Standard Deviation	Range
Spermatozoa mass activity	++++	++++
Progressive spermatozoa motility (%)	84.64±1.38	75 - 90
Spermatozoa Concentration ($\times 10^9$/ml)	1.91±0 .00	1.42 - 2.10
Spermatozoa Liveability (%)	96.91± 1.17	95 - 100

Table 4: Effect of insemination interval on fertility, hatchability and embryo mortality from laye rs breeders chicken using 0.02mL of undiluted semen for 5 weeks

Treatments (Insemination Interval)	Number of Egg Set	Fertility (%)	Embryo Mortality (%)				Hatch of Fertile (%)	Hatch of Egg Set (%)
			Early	Mid	Late	Total		
T1 (3 days)	286	52.65±7.25^{b}	9.77±2.10	10.81±3.35^{ab}	7.30±3.09	27.89± 6.08	72.11±6.80	39.65±8.48^{b}
T2 (6 days)	280	39.87±4.70^{ab}	18.48±5.26	5.49±2.14^{a}	3.08±1.30	27.05±5.15	72.95±5.15	29.34±4.77^{ab}
T3 (9 days)	208	22.98±5.71^{a}	17.57±8.99	25.30±7.06^{b}	1.00±1.00	43.87±6.0.6	58.13±5.88	14.50±4.94^{a}
T4 (12 days)	251	36.14±6.89^{ab}	11.54±3.82	14.89±4.71^{ab}	2.92±1.82	29.35±3.55	70.65±3.55	24.77±4.26^{ab}

$^{a,\,b}$: Means on the same column with different superscripts differ significantly (P<0.05). SE = Standard error

Discussion

The results of semen characteristics of pooled semen inseminated revealed that 0.02, 0.04, 0.06 and 0.08mL of raw semen inseminated contained 20.43×10^6, 40.87×10^6, 61.30×10^6 and 81.74×10^6 motile sperm cells, respectively. The non significant difference in the fertility across the treatments up to week four post insemination observed in this study contradicted the report of Saleh *et al.* (2012) and Tabatabaei (2010) that the maximum fertility of eggs was achieved with the use of 50 and 100 $\times10^6$ sperm cells, respectively, compared with least dose of 20.43×10^6 motile sperm cells used in this study. This also contradicted the report by Bakst *et al.* (2010) that the duration of fertility was directly related to the number of SST in their respective utero-vaginal junction folds. However, this result was in agreement with the observation of McCartney (1952) that there was no significant difference in fertility with undiluted semen doses ranging from 0.01mL to 0.05mL in turkeys. Moreover, no advantage was obtained in inseminating more than 0.02mL semen dose. Continual increase in the number of spermatozoa inseminated did not increase the level of fertility. The 0.02mL which was the least volume of semen inseminated still had highest fertility at week 4 among other treatments. This report therefore suggest that insemination of 20.43×10^6 sperm cells have fertile period of more than 28 days. Continue increases in the numbers of spermatozoa inseminated did not increase the level of fertility significantly. Total and early embryo mortality in week 3 is significantly higher in inseminated dose of 0.02mL than in other treatments (0.04mL, 0.06mL, 0.08mL and natural mating). This confirmed an observation made by Bramwell (2002) that old and stale sperm in the oviduct is associated with poor embryonic death. The hatch of fertile in natural mating (T5) that was significantly higher at week 2 than other treatments (T1, T2, T3 and T4) negates the observation by Surai *et al.*, (1996) and Hocking and Bernared, (1997). This result also contradicted the finding of Robinson (1996) who reported that when artificial insemination is practiced, hatchability percentage are increased compared to natural mating. The low hatch of fertile observed in birds inseminated with 0.04mL and 0.06mL could be as a result of increased in total embryo mortality. The hatch of fertile slowly decreased and reached zero in week 4 and this result is different from the report of Tabatabei *et al.* (2009) that total hatch rate decreased to zero in 16 days. The effective duration of insemination is the period post artificial insemination during which a hen lays 100% fertile eggs. This ascertained that hens inseminated demonstrated effective duration of fertile eggs at days 3 and 5, respectively. Observation from this study was similar to the report by Tabatabei *et al.* (2009) that maximum fertility was constant till day 5. The duration of fertile period in the birds inseminated with 0.02, 0.04, 0.06, 0.08 mL and natural mating were 24, 16, 18, 19 and 22 days, respectively. The short fertile duration in excess dose of 0.02mL can be as a result of old age of the hen and migration of high number of spermatozoa out of the SST to the infundibulum leading to fast dropped in fertility. Donoghue and Wishart (2000) stated that mechanism of sperm storage and slow release assured a succession of fertilised eggs in the absence of repeated copulation or artificial insemination.

The duration of fertility is the number of days during which a hen lays fertile eggs after a single artificial insemination and is determined largely by the sperm storage duration. Maximum fertility was 70.35%, 81.67% and 81.05% at days 2, 3 and 5 respectively in this study. This can be

suggested as efficient duration of fertile period for Nera black layer breeder hen. Romanoff and Romanoff (1963) suggested that maximum duration is a period up to oviposition of the last fertile eggs. Fertility dropped to 8.33% at day 25 and this can be assumed to be the maximum duration of fertile period. The maximum fertility and the duration of fertile period in this experiment was at variance with the report of Tabatabei *et al*. (2009) that maximum fertility in this study was obtained at day 3 while the duration of fertility was obtained at day 19.

The result from the first experiment revealed that semen dose of 0.02mL still showed fertility at day 28 and this informed the choice for semen dose in this experiment. The choice of interval used for this experiment was based on observation in experiment 1 that fertility was still economical around day 11. Significantly higher fertility recorded in 3 days insemination interval compared to other treatments in this result was similar to that of Resende *et al*. (1976) and McCartney (1976) that obtained higher fertility when insemination was done twice a week than weekly in fowls and chickens. However, Bratte and Ibe (1989) suggested weekly insemination with 0.04 mL undiluted semen would be most adequate for optimum fertility under the prevailing tropical conditions. The hatch of fertile across the treatments was similar. Hatch of set eggs in 9 days interval is relatively lower across group and this can be as result of high embryo mortality recorded. The relatively low mid embryo mortality in 6 days insemination interval than in 9 days interval can probably be as result of low number of stale sperm cells present before refilling the SST.

Conclusion

This study showed that inseminating semen dose of 0.02mL containing approximatley 20.43×10^{6} motile sperm cells in Nera black breeder layer chickens gave a maximum fertile period of 5 days. Insemination interval of 3 days using 0.02mL of semen gave optimum fertility level, embryonic survival, hatch of fertile and hatch of set eggs. Artificial insemination of semen dose of 0.02mL with 3 days interval are recommended to be practiced on farm insemination practices for Nera black breeder chickens. It is further recommended that further research should be conducted by using higher semen dose with varies days of insemination.

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