Research Article/Artículo de investigación

FERTILITY RESPONSE OF INDIGENOUS TURKEY HENS TO SEMEN DOSAGE AND OVIDUCTAL SPERMATOZOA STORAGE

RESPUESTA FÉRTIL DE PAVAS CRIOLLAS TURCAS A DOSIS SEMINALES Y AL RESERVORIO OVIDUCTAL DE ESPERMATOZOIDES

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ABSTRACT

Fertility response of indigenous turkey to oviductal spermatozoa storage using duration of fertile period, and the effect of undiluted semen dosage on fertility and embryo mortality was assessed. Sixty white-feathered local turkey hens were grouped into five treatments of twelve hens each. Semen was harvested from seventeen toms, pooled and inseminated into hens in groups one to four each with 0.02 mL ($SD_{0.02}$), 0.04 mL ($SD_{0.04}$), 0.06 mL ($SD_{0.06}$) and 0.08 mL ($SD_{0.08}$) of undiluted semen, containing approximately $80x10^6$, $160x10^6$, $240x10^6$ and $320x10^6$ motile sperm cells, respectively. Hens in group five (NM) were mated with three other toms at a mating ratio of one tom to four hens. Insemination and mating were done once daily for two successive days only, after which eggs were collected and incubated weekly for 10 weeks. Fertility and embryonic mortality were monitored weekly. Average fertility at the first three weeks of oviductal sperm age in $SD_{0.02}$ (94.2%), $SD_{0.04}$ (95.1%), $SD_{0.06}$ (98.0%) and $SD_{0.08}$ (93.8%) were significantly higher than NM (70.4%, $p<0.05$). Total embryo mortality was significantly ($p<0.05$) higher in $SD_{0.08}$ (26.2%) than $SD_{0.02}$ (11.9%), $SD_{0.04}$ (11.6%) and NM (6.2%) but similar to $SD_{0.06}$ (17.9%). The efficient duration of fertile period of the turkey hens was three weeks while their maximum duration of fertile period was eight weeks. Semen insemination dosage of 0.02mL containing approximately $80x10^6$ motile spermatozoa was an economic dose for insemination while $320x10^6$ (group $SD0_{.08}$) reduced embryo survival.

Keywords: Semen dose, artificial insemination, oviductal sperm storage, turkey fertility, fertile period.
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RESUMEN

Se midió la respuesta de pavas criollas al reservorio oviductal de espermatozoides, evaluando el efecto de la duración del periodo fértil y de la dosis de semen no diluido sobre la fertilidad y la mortalidad embrionaria. Sesenta pavas criollas de raza blanca fueron agrupadas en cinco tratamientos de doce pavas cada uno. El semen se obtuvo de siete pavos, este se mezcló y fue inseminado sin diluir, en pavas en grupos de una a cuatro para cada una de las siguientes dosis 0.02 mL ($SD_{0.02}$), 0.04 mL ($SD_{0.04}$), 0.06 mL ($SD_{0.06}$) and 0.08 mL ($SD_{0.08}$), conteniendo $80x10^6$, $160x10^6$, $240x10^6$ and $320x10^6$espermatozoides motiles, respectivamente. Las pavas en el grupo cinco (monta natural) fueron apareadas con tres pavos a razón de cuatro pavas por pavo. Tanto las inseminaciones como las montas se realizaron una vez al día y solo por dos días consecutivos, luego de este periodo los huevos fueron recolectado e incubados semanalmente por diez semanas. La fertilidad y la mortalidad embrionaria se monitorearon semanalmente. La fertilidad promedio durante las tres primeras semanas de edad del reservorio espermático oviductal en los grupos $SD_{0.02}$ (94.2%), $SD_{0.04}$ (95.1%), $SD_{0.06}$ (98.0%) and $SD_{0.08}$ (93.8%) fue significativamente más altas que en el grupo de NM (70.4%, $p<0.05$). La mortalidad embrionaria total fue significativamente mayor en el grupo $SD_{0.08}$ (26.2%, $p<0.05$) que en los grupos $SD_{0.02}$ (11.9%), $SD_{0.04}$ (11.6%) y NM (6.2%), pero similar a la del grupo $SD_{0.06}$ (17.9%). La duración eficiente del periodo fértil en pavas fue de tres semanas mientras que la duración máxima fue de ocho semanas. La dosis de inseminación de 0.02mL conteniendo aproximadamente $80x10^6$ espermatozoides motiles fue una dosis económica para la inseminación, mientras que la dosis conteniendo $320x10^6$espermatozoides motiles (grupo $SD_{0.08}$) redujo la sobrevivencia embrionaria.

Palabras clave: dosis seminal, inseminación artificial, reservorio espermático oviductal, fertilidad en pavas, periodo fértil.
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INTRODUCTION

Artificial insemination (AI) is employed in turkeys, especially in improved breeds to ameliorate the challenge of low fertility that may arise from difficulty in mating and also to improve the breeding efficiency of the toms. Christensen et al. (2005) observed that breed difference may cause differences in fertility and therefore concluded that genetic selection for economically important traits may affect sperm hydrolysis of the perivitelline layer and subsequent fertility and embryo viability. Brillard (1992) also reported that strain, age or semen dose can affect oviductal sperm storage, though strain effects were observed to be higher. This implies that the lower sperm concentration of semen from the local turkeys, when compared to the exotic strains (Zaharadeen et al., 2005) may also affect the semen dosage required for insemination.

There is a paucity of information on research in AI in the indigenous turkeys in Nigeria (Yahaya et al., 2013). Related studies are limited to semen evaluation (Zaharadeen et al., 2005; Ngu et al., 2013) and preliminary AI studies (Yahaya et al., 2013). Adopting AI in the indigenous turkeys in Nigeria will help maximize their potentials and serve as a basis for their improvement as AI is known to be a vital tool in the improvement of animal productivity. A step in establishing AI schedule for the local turkeys is to determine their sperm storage potential and the minimum semen dose required for optimal fertility.

The time a female animal is inseminated naturally or artificially does not always coincide with ovulation time in avian species. Therefore, to increase chances of fertilization, sperm cells are naturally stored in the oviduct to ensure oocytes are encountered with them at the right time and place (Matsuzaki et al., 2014). This storage period however varies among and within species. While some reptiles can store sperm cells for up to seven years (Holt and Lloyd, 2010), birds for some weeks (Bakst et al., 2010), mammals may only store theirs for some days (Holt, 2011).

For sustained fertility during repeated inseminations, it is important to know for how long each species or strains within, can store sperm cells that will ensure maximum fertility. It is also necessary to know if this duration is affected by the number of sperm cells inseminated. The period of oviductal sperm storage is identified as the 'fertile period' in poultry and generally reflects the duration of fertility (Lake, 1975; Bakst et al., 1994). The day after insemination till the last fertile egg or two consecutive infertile eggs has been described as the maximum duration of fertile period in poultry by Liu et al. (2008). They described an efficient duration of fertile period as the period after insemination till the first infertile egg and suggested this as a measure of the sperm storage potential of a hen. However, this has not been reported in indigenous turkeys in Nigeria. Therefore, this study was designed to assess the oviductal sperm storage potential of indigenous turkeys in Nigeria using duration of fertile period and the effect of semen dosage on fertility, embryo mortality and hatch of set eggs.

MATERIALS AND METHODS

The research was carried out at the poultry unit of the Teaching and Research Farm and the Animal Physiology Laboratory, Department of Animal Science, of the University of Ibadan, Ibadan, Nigeria. White-feathered indigenous turkeys (*Meleagris gallopavo*) were sourced locally from a turkey breeder in Ibadan, Oyo state. Twenty-two toms were trained for semen collection twice weekly at about eight months of age using the abdominal massage technique (Burrows and Quinn, 1937).

Experimental Layout

Twenty toms selected at 10 months old and weighing 8.06±0.76kg and sixty hens of 8 months old with weight, 3.46±0.33kg were used for the study. Three toms, with sperm concentration close to the mean were separated for natural mating from the twenty toms. All the toms were maintained on deep litter while the hens were kept in cages. The cage dimension was 122 x 76 x 91 cm (length x breadth x height) housing four hens each. They received turkey breeder ration containing 2,900Kcal of metabolizable energy and 17% crude protein. The hens were divided into five treatment groups of three replicates with four hens per replicate in a completely randomized design. The four hens comprising of a replicate were housed together in a cage unit. Hens were not mated or inseminated prior to the commencement of the study to ensure there were no sperm cells in the oviduct in preparation for the experiment.

Semen collection and insemination

Semen was harvested from Seventeen toms and pooled into a collection tube. The period the semen flowed down the phallus gave enough time for a quick assessment, as semen from all the toms were collected together in a collection tube due to its viscosity. Semen

contaminated with faecal matter or that showed poor quality from colour or viscosity was discarded. The pooled semen was gently stirred for uniformity. The collection tube was wrapped with dry cotton wool to minimize the influence of external temperature on semen. A portion of the pooled semen was evaluated for progressive sperm motility (subjective scoring method), sperm concentration (Neubauer haemacytometer method) and sperm liveability (eosin–nigrosin staining method) all as described by Ewuola and Egbunike (2010).

Hens in groups 1 ($SD_{0.02}$), 2 ($SD_{0.04}$), 3 ($SD_{0.06}$) and 4 ($SD_{0.08}$) were inseminated with the pooled semen immediately after collection at doses of 0.02mL, 0.04mL, 0.06mL and 0.08mL, respectively. The process of insemination was done as described by Burrows and Quinn (1939). The oviduct of each hen was everted and semen was deposited into it at a depth of about 2.5 cm using a graduated tuberculin syringe with a glass rod attached to it. As soon as the required dose of semen was expelled through a light pushing pressure on the syringe, the pressure on the abdomen was released to suck in the semen and return oviduct to its position. The process of semen collection and insemination of all the hens did not exceed 45 minutes. Hens in group five (NM) were mated naturally on the floor with the three toms separated for mating at a ratio of one tom to four hens representing each replicate. Mating was supervised and a single mounting and ejaculation was allowed for each hen after which it was placed in the cage.

The insemination / mating were done for two successive days only and not repeated. This was to ensure only sperm cells introduced into the oviduct were stored in it during the period of egg collection. They were also done after 5pm to minimize the presence of an egg in the oviduct. The post insemination / mating period was regarded as the oviductal sperm age as this implied the period the sperm cells had stayed in the oviduct.

Fertility, embryo mortality and hatch assessment

The day after the second insemination marked the day for the first egg collection. Eggs were collected daily from each treatment group, marked and stored at a temperature of 24°C to 26°C and relative humidity of 70% to 85% for a week. Incubation of eggs was done weekly for 10 weeks following hatchery protocols. Egg candling was done on day 25 of incubation and all candling clears were removed. Eggs with evidence of developing embryo were transferred into the hatching unit. Hatching took place at day 28 and all unhatched eggs and candling clears were broken–out to separate infertile eggs from those fertile but with embryonic deaths. Infertile eggs were those eggs which upon break-out, were devoid of any form of embryonic mass. Eggs that had a form of embryonic mass upon break-out were categorized as fertile eggs with embryo mortality. Embryonic deaths were classified as early (occurring at the 1st week of incubation), mid, at the 2nd and 3rd week while late embryonic mortality was at the 4th and last week of incubation (Fairchild et al., 2002). Eggs with embryo mortality at piping were classified as late embryo deaths. Fertility was expressed as a percentage of set eggs, while each category of embryo mortality was expressed as a percentage of fertile eggs. Total hatch of set was the total hatched poults expressed as a percentage of the total eggs set.

Data Collection and Statistical Analysis

Data was collected on post insemination / mating weekly egg fertility, embryo mortality and hatch of set eggs. The duration of fertile period was also noted. Analysis of variance was done on square root transformed data using general linear model of SAS (Statistical Analytical System, 2003) and means were separated using Duncan's multiple range test.

RESULTS

The mean spermatozoa characteristics of pooled semen inseminated on the two successive days are presented in Table 1. Sperm concentration was $4.48\pm0.26x10^9$ cells/mL, livability was $93.53\pm3.57\%$ while progressive spermatozoa motility was $92.5\pm2.89\%$. This implied that 0.02 mL, 0.04 mL, 0.06 mL and 0.08 mL semen inseminated contained approximately $80x10^6$, $160x10^6$, $240x10^6$ and $320x10^6$ motile sperm cells, respectively.

The effect of semen dose on post – insemination / mating weekly fertility levels is presented in Table 2. During the first three weeks after insemination, percentage fertility was similar among the inseminated groups. It ranged from $90.6\pm2.4\%$ to $100.0\pm0.0\%$ and was significantly ($p<0.05$) higher than NM ($70.4\pm93.4\%$). Fertility in $SD_{0.06}$ was significantly ($p<0.05$) lower than $SD_{0.04}$ and $SD_{0.08}$ at the 4th week. Significant differences were not observed among all groups at subsequent weeks. They were all infertile between the 8th and 9th week.

Table 1: Characteristics of Pooled Turkey Semen Inseminated on Two Successive Days

Semen Parameters	Mean Values (± Standard Deviation)	Range
Spermatozoaconcentration (x10^9 cells/mL)	4.48±0.26	4.20 - 4.81
SpermatozoaLiveability (%)	93.53±3.57	87.50 - 97.26
ProgressiveSpermatozoaMotility (%)	92.50±2.89	90.00 - 95.00

Table 2: Fertility (%) of Eggs from Turkey Hens Inseminated with Different Semen Doses (Mean±SE)

Weekspost Insemination	Semen dose				
	0.02 mL ($SD_{0.02}$)	0.04 mL ($SD_{0.04}$)	0.06 mL ($SD_{0.06}$)	0.08 mL ($SD_{0.08}$)	Natural Mating (NM)
1	95.6 ±2.6 ^{a}	94.3 ±0.7 ^{a}	98.3 ±1.7 ^{a}	98.6 ±1.4 ^{a}	71.8 ±3.6 ^{b}
2	95.8 ±4.2 ^{a}	97.4 ±2.6 ^{a}	100.0 ±0.0 ^{a}	90.6 ±2.4 ^{a}	68.5 ±9.3 ^{b}
3	91.1 ±4.5 ^{a}	93.8 ±6.3 ^{a}	95.6 ±4.4 ^{a}	92.1 ±4.8 ^{a}	71.1 ±6.3 ^{b}
4	75.5 ±5.2 ^{ab}	83.1 ±8.6 ^{a}	58.9 ±4.8 ^{b}	79.9 ±4.2 ^{a}	72.6±1.2 ^{ab}
5	63.4 ±1.9	58.2 ±10.0	56.7 ±8.8	65.1 ±8.3	45.8 ±4.1
6	17.9 ±9.0	22.9 ±2.9	20.2 ±11.6	17.0 ±5.2	20.0 ±6.7
7	3.3 ±3.3	7.8 ±4.2	4.2 ±4.2	3.0 ±3.0	7.4 ±7.4
8	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	4.2 ±4.2
9	0.0 ±0.0	3.6 ±3.6	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
10	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0

a, b – means across same row with different superscripts are significantly different ($p<0.05$); SE - Standard Error

Fertility was above 90% in the first 3 weeks after insemination but declined significantly ($p<0.05$) to 74.3±13.1% at the fourth week, 60.9±12.3% at the fifth week, 19.4±11.9% at the sixth week, 4.6±5.8% at the seventh, and finally down to 0.0±0.0% at the eight week. The efficient duration of fertile period was therefore three weeks while the maximum duration was eight weeks.

The duration of fertile period and the effect of Oviductal Spermatozoa (OS) age on fertility of the artificially inseminated groups of hens are presented in Figure 1. Fertility declined in the hens as the stored spermatozoa stayed longer in the oviduct.

The effect of insemination dose on fertility, embryo mortality and hatch of set eggs is shown in Table 3. Results were combined for the first 3 weeks after insemination because this was the period when maximum fertility was recorded. Early Embryo Mortality (EEM) was significantly ($p<0.05$) higher in $SD_{0.08}$ than $SD_{0.02}$, $SD_{0.04}$ and NM but similar to $SD_{0.06}$. The Mid Embryo Mortality (MEM) in $SD_{0.08}$ was significantly ($p<0.05$) higher than NM while both were similar to $SD_{0.02}$, $SD_{0.04}$ and $SD_{0.06}$. Late Embryo Mortality (LEM) was similar across the groups. Total embryo mortality (TEM) was significantly ($p<0.05$) higher in $SD_{0.08}$ than $SD_{0.02}$, $SD_{0.04}$ and NM, while $SD_{0.06}$ was similar to all other groups. The hatch of set eggs was significantly higher in $SD_{0.02}$, $SD_{0.04}$ and $SD_{0.06}$ compared to $SD_{0.08}$ and NM. Results on embryo mortality were not presented for subsequent weeks because of the low number of fertile eggs at this period. The fact that embryo mortalities are expressed as a percentage of fertile eggs, low egg number would therefore have resulted in a large error margin.

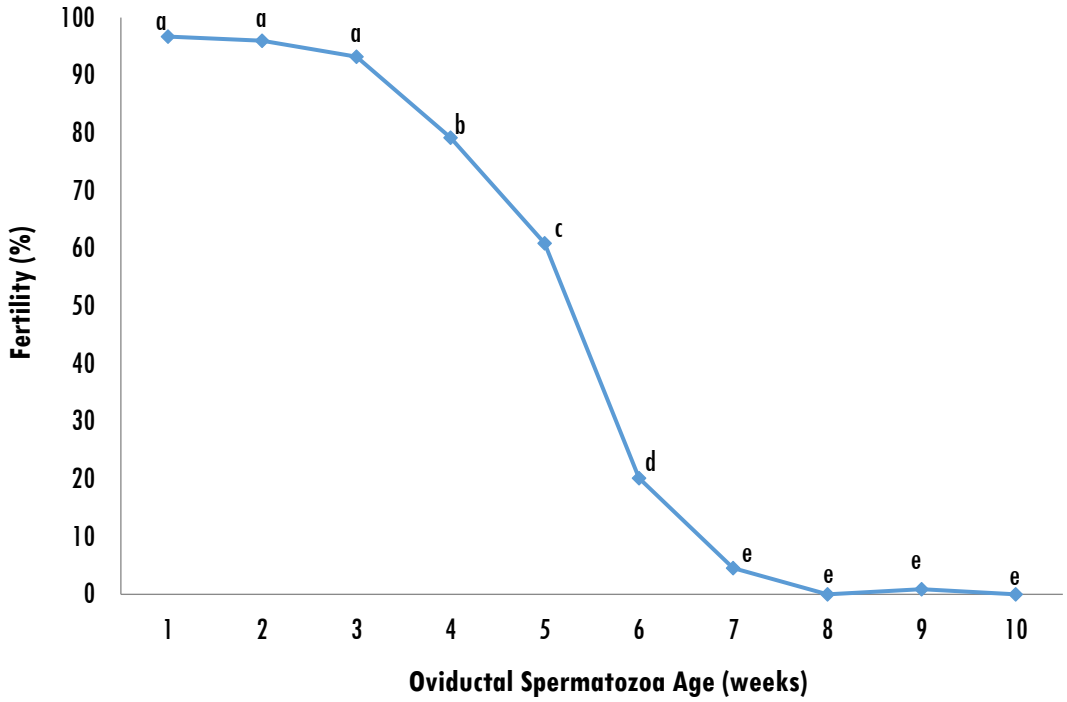

Table 3: Egg Fertility, Embryo Mortality and Hatch of Set Eggs from Turkey Hens at the 1st, 2nd and 3rd Week after Insemination with different Semen Doses (Mean±SE)

Semen Dose Inseminated (mL)	Fertility (%)	EEM (%)	MEM (%)	LEM (%)	TEM (%)	Hatch of Set Eggs (%)
0.02 ($SD_{0.02}$)	94.2±2.1^{a}	4.3±1.4^{b}	3.3±1.8^{ab}	4.3±1.4	11.9±2.7^{bc}	83.2±3.8^{a}
0.04 ($SD_{0.04}$)	95.1±2.0^{a}	4.1±1.3^{b}	0.9±0.9^{ab}	6.6±1.9	11.6±3.1^{bc}	84.1±3.3^{a}
0.06 ($SD_{0.06}$)	98.0±1.5^{a}	7.1±2.9^{ab}	1.5±1.0^{ab}	9.3±3.8	17.9±4.2^{ab}	80.1±3.6^{a}
0.08 ($SD_{0.08}$)	93.8±2.0^{a}	12.1±2.3^{a}	4.0±1.1^{a}	10.1±3.5	26.2±3.8^{a}	69.4±4.2^{b}
Natural Mating	70.4±3.4^{b}	2.0±1.3^{b}	0.0±0.0^{b}	4.1±2.2	6.2±3.4^{c}	65.6±3.1^{b}

a, b, c – means along same column with different superscripts are significantly ($p<0.05$) different; SE- Standard Error; EEM – Early Embryo Mortality, MEM – Mid Embryo Mortality, LEM – Late Embryo Mortality

DISCUSSION

The duration of fertile period in a hen is a function of the ability of its sperm storage tubules to store sperm cells. It has been observed that variations exist in the duration of fertile period among poultry species (Bakst et al., 2010). It was assumed in this study, that the multiplicative dose of semen would mean more sperm cells available for storage in the sperm storage tubules (SST). Therefore, higher fertility levels could have being sustained for a longer period after insemination or the duration of fertile period may be lengthened. This assumption differs from the results obtained in this study (Table 2). Compton and Van Krey (1979) suggested that only a finite number of

sperm cells are capable of entering the SST within a given period. Despite the multiplicative semen dose administered across the treatment groups, the absence of a significant difference among the inseminated groups suggests that insemination in excess of 0.02mL (approximately $80x10^6$ motile cells) is probably more than the maximum number of spermatozoa which can be accepted by the SST at a given period. Thus, increasing the dose therefore resulted in semen wastage. This result is also partly supported by reports from Brillard (1993), that frequent inseminations with moderate number of sperm cells should be more efficient than larger doses at longer intervals. The relatively lower fertility from the naturally mated hens may be an indication that the volume of semen ejaculated by the toms into the female genitalia did not contain enough sperm cells that was sufficient to fill the SST. The reduced volume may have resulted from semen wastage from improper cloacal contact, or the actual volume ejaculated naturally was low, especially since only one ejaculation was allowed for each of the two consecutive days. This further confirms reports from Mohan et al. (2018) that in poultry species, better fertility can be obtained through AI with good quality semen than natural mating.

The results on the maximum duration of fertile period is similar to that reported by Bakst (1988) who observed 8 to 9 weeks for large white turkey hens inseminated with 75 million and 200 million sperm cells for two successive days. Although, his results indicated that fertility levels were still as high as 88.1% to 98.0% at 5 weeks before, a sharp drop to 0% at the 8^{th} to 9^{th} week post insemination. The reason for the high fertility sustained for a longer period may be due to differences in breed, or the fact that the semen inseminated in that study was diluted (with Beltsville poultry semen extender) while in this study, undiluted semen was used. This may probably have been a medium that sustained the cells better in the storage tubules. However this only seemed to sustain high fertility for longer and not lengthen the duration of fertile period. Despite the assumed low volume of semen in the naturally mated hens, their duration of fertile period was similar to the inseminated groups. This therefore could be an indication that the maximum duration of fertile period is more related to the oviductal sperm age rather than the number of spermatozoa in the SST once probably a threshold is reached.

The results presented in Figure 1 is a weekly mean fertility of all hens artificially inseminated ($SD_{0.02}$ to $SD_{0.08}$) to assess the effect of oviductal sperm age on fertility and the sperm storage potential of the hens. Maximum fertility of 93.1% to 96.7% observed at the first 3 weeks and without significant differences among the weeks considered it an efficient duration of fertile period as described by Liu et al. (2008). They reported it as a better way to assess the sperm storage potential of a hen. Although efficient duration of fertile period was described as the day after insemination to the first infertile egg observed, this can also be assumed to be the period of maximum fertility. As such, the period of maximum fertility was considered as the efficient duration of fertile period in this study. Fertility reached zero at the 8^{th} week. It was therefore observed, that efficient duration of fertile period was three weeks, while the maximum duration of fertile period was eight weeks in the indigenous turkey hens.

Physiological polyspermy has been reported to be normal in avian fertilization process (Hemmings and Birkhead, 2015). However, when abnormally high number of sperm cells reaches the site of fertilization, there is an increase in the possibility of pathological polyspermy and a high incidence of early embryo mortality (Bakst, 1998). There is the possibility that the significantly higher Early Embryo Mortality (EEM) observed by hens in the group with the highest semen dose ($SD_{0.08}$) when compared with those with lower doses could have been as a result of excessive sperm number at the site of fertilization. This is more likely because EEM is the stage of mortality associated with pathological polyspermy as reported by Bakst (1998). Also, EEM of 12.1% recorded in $SD_{0.08}$ is higher than that reported by Fairchild et al. (2002) which were 4.9% and 7.5% in old and young turkey hens respectively. Mroz et al. (2007) reported that the peak of embryo mortality observed in turkeys is between days 3 and 6 and the 2^{nd} between days 26 and 28 of incubation. The period in between, which is classified as the period of Mid Embryo Mortality (MEM) is rarely seen except in cases of embryo malformation or nutritional disorder (Abudados, 2010). Therefore, the significant difference between $SD_{0.08}$ and NM in MEM further suggests that the cause of the mortality may be due to excessive sperm cells at the site of fertilization which may have resulted in embryo malformation and deaths. Moreover, since the hens that were naturally mated were assumed from results of this study to have had the least semen volume introduced into their oviduct. This result therefore shows that insemination of up to 320 million cells reduced embryo viability. The significantly lower hatch of set observed in $SD_{0.08}$ and NM was due to the higher total embryo mortality observed in $SD_{0.08}$ and the lower fertility in NM since the primary determinants of hatch are fertility and embryo mortality.

CONCLUSIONS

Based on results obtained from this study, the indigenous turkey hens have an efficient duration of fertile period of at least 3 weeks. This implies that their oviduct has the capacity to support sperm storage for maximum fertility for at least 3 weeks post insemination while their maximum duration of fertile period was 8 weeks. Semen insemination dose of 0.02mL containing approximately $80x10^6$ sperm cells can be considered as an economic dose for insemination of undiluted semen, while a dose containing sperm cells of up to $320x10^6$ reduced embryo

survival.

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