EFFECT OF SEMEN DILUTION RATIO AND INSEMINATION INTERVALS ON TURKEY HEN FERTILITY AND EMBRYO VIABILTY

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ABSTRACT
The fertility and embryo mortality of indigenous turkeys in response to varied semen dilution ratios for weekly and fortnight inseminations were assessed. In a 2x4 factorial arrangement of a randomized complete block design, 64 local turkey hens were divided into eight groups of eight hens each. Each group of hens comprised four replicates of two hens each. Hens from two replicates were 9 months old while the other two were 16 months old. Semen was pooled from 10 turkey toms. Hens were inseminated with the pooled semen diluted with normal saline at a ratio of 1:0 (T1:0), 1:1 (T1:1), 1:2 (T1:2) and 1:3 (T1:3) containing approximately 90x106, 45x106, 30x106, 22.5x106 motile spermatozoa, respectively, weekly and fortnightly over a period of 12 weeks. Fertility, hatch parameters and embryo mortality were assessed. Data were analysed using ANOVA at α0.05. Fertility at weekly and fortnight inseminations were similar (80.8±1.6% and 81.8±1.6%, respectively), but was significantly (p<0.05) lower in T1:3 (74.8±2.3%) than T1:0 (87.1±2.2%) and T1:2 (85.3±2.3%) among the dilution ratios. However, with the interaction of the two factors, the only significance observed was in fortnight insemination with T1:3 (72.8±3.1%), which was significantly (p<0.05) lower than T1:0 (89.3±3.1%) and T1:2 (86.8±3.3%). Hatchability and embryo mortality was similar across all groups. The block effect was not significant across the parameters. Weekly or fortnight insemination intervals with semen diluted at 1:2 or containing approximately 30x106 motile spermatozoa was sufficient to give optimal fertility without compromising embryo viability in the indigenous turkey hens.
Keywords; Tom semen, Dilution ratio, Insemination intervals, Turkey fertility, Embryo viability.

INTRODUCTION

Turkey semen is known to have relatively high spermatozoa concentration among other poultry species (Bramwell, 2021). This attribute can be maximised to reduce the number of males required for breeding and increase their reproductive efficiency. Adebisi and Ewuola (2019a; 2019b) observed that fortnight insemination of turkeys using undiluted semen gave optimal fertility, while dilution at a ratio of 1 to 1 with normal saline was equally recommended. However, the extent to which this semen can be diluted needs to be assessed relative to the frequency of insemination without compromise on the fertility or embryo mortality. In addition, the costs associated with artificial insemination can be justified with the frequency of insemination of the hens and efficient use of toms. This study therefore seeks to assess the effect of semen dilution ratio or number of spermatozoa inseminated and insemination intervals on fertility, hatch parameters and embryo mortality of indigenous turkeys.

MATERIALS AND METHODS

The study was carried out at the Poultry unit of the Teaching and Research Farm and Animal Physiology Laboratory, University of Ibadan, Ibadan. Ten turkey toms of 18 months old and 64 hens were used for the study. The hens were randomly divided into eight treatment groups of four replicates with two hens each. The experimental design was a randomised complete block design in a 4 x 2 factorial arrangement. The factors were semen dilution ratio and insemination intervals, while the block effect was hen age; old hens (16 months) and young hens (9 months). Semen was pooled from the toms and prior to insemination, a portion was set aside for semen quality assessment as described by Ewuola and Egbunike (2010). Hens in groups 1 to 4 were inseminated with the pooled semen diluted with normal saline at a ratio of 1:0 ($T_{1:0}$), 1:1 ($T_{1:1}$), 1:2 ($T_{1:2}$) and 1:3 ($T_{1:3}$) at Weekly Intervals (WI), while those in groups 5 to 8 were inseminated at Fortnight Intervals (FI) with the same varied dilution ratios as with groups 1 to 4. Insemination and weekly egg collection for incubation was done for 12 weeks. Fertility, embryo mortality, hatch parameters were determined as described by

Adebisi and Ewuola (2019a). Data was analysed using analysis of variance (on square root transformed data) using general linear model of SAS (2003). Means were separated using Duncan's multiple range test of same software.

RESULTS AND DISCUSSION

The mean characteristics of pooled semen prior to dilution and insemination over 12 weeks are presented in Table 1. This implied that $T_{1:0}$, $T_{1:1}$, $T_{1:2}$ and $T_{1:3}$ contained approximately $90x10^6$, $45x10^6$, $30x10^6$ and $22.5x10^6$ motile sperm cells, respectively per 0.02mL insemination dose.

Table 1: Mean Characteristics of Weekly Pooled Semen Prior to Dilution and Insemination over Twelve Weeks

	Spermatozoa concentration ($x10^9$ cells/mL)	Progressive spermatozoa motility (%)	Spermatozoa liveability (%)
Mean values	5.37±0.56	86.39±4.86	94.83±1.87
Range	4.29 – 6.12	80.00 – 95.00	91.30 – 97.40

Table 2: Effects of Semen Dilution Ratio, Insemination Intervals and their Interactions on Fertility and Hatch Parameters of Eggs from Turkey Hens (Mean±SE)

			Parameters measured (%)		
Factors	**Levels**		**Fertility**	**Hatch of fertile eggs**	**Hatch of set eggs**
Insemination intervals	1 week		80.8±1.6	84.6±1.8	68.2±2.0
	2 weeks		81.8±1.6	84.3±1.7	69.3±2.0
P – Value			0.7512	0.8889	0.7116
Semen dilution ratio	1:0		87.1±2.2^{a}	82.3±2.5	72.4±2.8^{a}
	1:1		78.2±2.2^{bc}	83.9±2.5	65.8±2.8^{ab}
	1:2		85.3±2.3^{ab}	86.2±2.5	73.4±2.8^{a}
	1:3		74.8±2.3^{c}	85.5±2.4	63.4±2.8^{b}
P – Value			0.0002	0.6995	0.0257
Insemination intervals X Semen dilution ratio	1 week	1:0	84.9±2.8^{ab}	83.8±3.4	70.7±3.7
		1:1	78.0±3.3^{ab}	83.2±3.8	65.4±4.2
		1:2	84.2±3.2^{ab}	87.5±2.9	73.6±3.8
		1:3	76.7±3.4^{ab}	83.9±3.1	63.7±3.5
	2 weeks	1:0	89.3±2.6^{a}	81.4±3.9	74.4±3.9
		1:1	78.3±2.5^{ab}	83.4±3.7	66.0±3.7
		1:2	86.8±3.0^{a}	83.9±3.3	72.1±3.8
		1:3	72.8±4.4^{b}	87.2±4.2	63.2±4.9
P – Value			0.570	0.8352	0.9290

a, b – means along same column with different superscripts are significantly different ($p<0.05$)

The effect of semen dilution ratio and insemination intervals and their interactions on fertility, hatchability and hatch of set eggs is presented in Table 2. There were no significant differences between WI and FI for all the parameters assessed. This result is in line with reports from Adebisi and Ewuola (2019a). However, fertility in $T_{1:0}$ was significantly ($p<0.05$) higher than $T_{1:3}$ among the dilution ratios. When the interactions of the two factors were considered, there were no significant differences among most of the groups except for fertility at FI in $T_{1:3}$ (72.8±3.1%), which was significantly ($p<0.05$) lower than $T_{1:0}$ (89.3±3.1%) and $T_{1:2}$ (86.8±3.3%). This may have been as a result of an overall lower number of inseminated sperm cells received by $T_{1:3}$. The Results on the dilution ratios at weekly inseminations are in line with recommendations from Sexton (1976) who reported that as few as 25 million sperm cells per weekly insemination were needed for optimum fertility in Turkeys. Christensen and Bagley (1989) suggested 50 million sperm cells weekly with an increase to 200 million as the hens grow older, while Bramwell (2021) recommended 150 - 300 million cells for diluted semen depending on the age of the hens. There seemed to be an increasing

requirement for sperm cells for insemination over the decades and this could be related to the genetic improvement of the birds over time.

Considering the period the study was conducted by Sexton (1976), the turkeys used at the time may have had little or no genetic advancement, hence its similarity with the random bred unimproved strains used in this study. Christensen *et al.* (2005) observed that the selection of turkeys for nearly 40 generations for increased 16-week body weight has decreased fertility and embryo survival compared with its random bred control line. The implication of this may mean more sperm cells requirement for insemination by the improved hens to attain optimal fertility. Breed differences may also cause differences in fertility. There are reports that bronze turkeys fertilised at a faster rate than that large white turkeys when inseminated with equal numbers of viable sperm cells (Christensen *et al.*, 2005). There were no significant differences ($p>0.05$) in the hatchability of the eggs, while the hatch of set eggs followed same pattern as that of fertility. The effect of the interaction of semen dilution ratio and insemination intervals on embryo mortality in turkeys is presented in Table 3.There were no significant ($p>0.05$) differences among the groups. Hemming and Birkhead (2015) suggested that under natural conditions, a minimum number of sperm cells must enter the ovum to ensure zygote formation and development. When too few cells penetrate, embryo survival is significantly reduced. The absence of significant differences in embryo mortality among the treatments therefore indicates that as few as 22.5 million motile sperm cells inseminated at 2 weeks intervals was sufficient to ensure fertilisation, embryo development and survival.

Table 3: Effects of Semen Dilution Ratio and Insemination Intervals on Embryo Mortality in Turkeys (Mean±SE)

		Embryo mortality (%)			
Insemination intervals	**Semen dilution ratio**	**EEM**	**MEM**	**LEM**	**TEM**
1 week	1:0	6.6±2.5	1.3±1.3	8.4±2.3	16.3±3.4
	1:1	7.1±2.6	0.0±0.0	7.1±2.1	14.2±3.1
	1:2	3.3±1.6	0.4±0.4	7.6±2.5	11.3±2.8
	1:3	4.8±2.0	0.6±0.6	10.7±2.6	16.1±3.1
2 weeks	1:0	3.7±1.5	2.1±1.4	12.9±3.9	18.7±3.9
	1:1	5.5±2.3	0.5±0.5	9.4±3.0	16.6±3.7
	1:2	3.2±1.7	1.8±1.4	10.3±2.7	15.3±3.3
	1:3	6.7±3.2	1.3±1.3	4.9±2.7	12.8±4.2
P – Value		0.6045	0.9385	0.2213	0.7723

Means among treatment groups are not significant ($P>0.05$) -; ; EEM – Early Embryo Mortality; MEM – Mid Embryo Mortality; LEM – Late embryo Mortality; TEM – Total Embryo Mortality

The block effect of hen age on the interaction of semen dilution ratio and insemination intervals on fertility and hatch parameters of eggs from turkey hens is presented in Table 4. There were no significant differences across most of groups for all the parameters. However, an apparent lower fertility observed for $T_{1;3}$ at F1 in older hens, which was significant only when compared to a few other groups across the treatments imply that the age of the hen may influence fertility when inseminated sperm cells are fewer and insemination interval is wider. This particular treatment groups received the least number of spermatozoa cells per unit time in addition to being older. It has been reported that when young and old hens were inseminated with same semen volume from same males, the rate of release of sperm cells from the sperm storage tubules of the older hens was nearly twice that of the younger hens (Brillard, 1993). The inability to maintain prolonged storage may be partially responsible for the lower fertility observed in the older flock (Bakst *et al.*, 1994). However, we may not be conclusive about the block effect of hen age on fertility in this study as the sample size may be too low judging by the number of hens representing each hen age group and the absence of significant difference among the factors.

CONCLUSION

Semen diluted up to a ratio of 1 to 2 or containing at least 30 million motile spermatozoa inseminated at weekly or fortnight intervals was sufficient to maintain optimal fertility in the locally adapted turkey hens over a period of 12 weeks without compromise on hatchability or embryo viability. Studies to determine the age effect on number of sperm cells required for insemination at specific intervals will require a larger sample size to minimize error of mean and give a more reliable result as it was not conclusive from this study.

Table 4: Block Effect of Hen Age on the Interaction of Semen Dilution Ratio and Insemination Intervals on Fertility and Hatch Parameters of Eggs from Turkey Hens (Mean±SE)

Block	Factors		Parameters (%)		
Hen age group	Insemination intervals	Semen dilution ratio	Fertility	Hatch of fertile eggs	Hatch of set eggs
Young Hens	1 week	1:0	84.5±3.9^{ab}	86.2±3.7	72.6±4.4
		1:1	79.0±4.8 ^{ab}	74.8±5.9	59.4±5.9
		1:2	74.5±5.3 ^{ab}	90.8±4.4	67.0±5.7
		1:3	80.4±5.1 ^{ab}	87.6±3.8	69.2±4.9
	2 weeks	1:0	86.9±4.4 ^{a}	76.3±6.4	69.8±6.3
		1:1	84.1±3.3 ^{ab}	80.7±5.1	68.5±5.4
		1:2	80.9±3.9 ^{ab}	79.1±4.4	64.0±4.7
		1:3	80.4±5.6 ^{ab}	84.9±5.2	67.4±6.3
Older hens	1 week	1:0	85.4±4.0^{ab}	80.0±6.3	67.9±6.4
		1:1	76.9±4.6^{ab}	93.2±3.3	72.5±5.6
		1:2	93.1±2.8^{a}	84.4±3.9	79.5±4.9
		1:3	73.0±4.5^{ab}	80.1±4.8	57.9±4.7
	2 weeks	1:0	91.7±2.7^{ab}	86.7±4.3	79.2±4.5
		1:1	72.6±3.3^{ab}	87.0±5.3	62.8±4.8
		1:2	92.7±4.1^{a}	90.4±4.6	83.1±5.2
		1:3	65.3±6.5^{b}	89.3±6.6	59.4±7.5
P-value			0.713	0.1226	0.1833

a, b - means along same column with different superscripts are significantly different ($p<0.05$)

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