EGG FERTILTIY AND EMBRYO VIABILITY OF TURKEY HENS INSEMINATED WITH VARIED SEMEN DILUTION RATIO AT DIFFERENT INTERVALS

Adebisi, K. A. & Ewuola, E. O.

Animal Physiology and Bioclimatology Unit, Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Corresponding Author: Adebisi, K. A.; Email: karamatsanusi@yahoo.co.uk; +2348023503018

ABSTRACT

When semen is diluted, the number of sperm cells inseminated is reduced and this reduction can affect fertility especially with the frequency at which insemination is done. The objective of this study was therefore to determine the effect of the interaction of semen dilution ratio and insemination intervals on turkey fertility and embryo viability. Local turkeys were used for the study, ten toms and sixty-four hens. The hens were randomly divided into eight groups of four replicates. In a 4x2 factorial arrangement of a randomized complete block design (old and young hens), two insemination intervals (one and two weeks) and four dilution ratios of semen to normal saline (1:0, 1:1, 1:2 and 1:3) were used in insemination. Eggs collected were incubated weekly for 10 weeks. Fertility and embryo mortality (early and mid) were assessed by breakout of candling clears, while unhatched eggs were considered late embryo mortality. Analysis of variance was done on square root transformed data at $\alpha_{0.05}$.

Results of the main effects indicated no significant difference in fertility between weekly or fortnight inseminations, however, dilution of 1:0 and 1:2 were significantly higher than 1:3. When the interaction of factors was observed, 1:0 (89.3±2.8%) and 1:2 (86.8±3.0) were significantly ($p<0.05$) higher than 1:3 (72.8±4.4%) at fortnight inseminations only, though values were not significantly different from all other groups. Embryo mortality was not affected by the interaction. Therefore, to maximize fertility in local turkeys, semen should not be diluted beyond 1:2 (minimum of $30x10^6$ motile spermatozoa) at fortnight insemination intervals.

Keywords: Embryo mortality, Hatchability, Insemination interval, Semen dilution, Turkey fertility

INTRODUCTION

The additional cost incurred on insemination needs to be justified with the frequency of insemination and optimum use of semen from the toms. This is expected to reduce the labour cost associated with insemination, minimize handling of birds and also, reduce the number of males kept for breeding purpose. Thus, increasing the dilution ratio of semen at a less frequent insemination, without compromise on fertility and hatchability is expected to achieve this goal. Adebisi and Ewuola (2019a) observed optimal fertility in local turkeys at two weeks insemination interval using undiluted semen. Normal saline was also reported as a suitable diluent, if insemination is done immediately after semen collection (Adebisi and Ewuola, 2019b). Turkey semen is relatively high in sperm concentration and viscosity when compared to other poultry species (Bramwell, 2012). Its dilution prior to Artificial Insemination (AI) is therefore very essential, to increase the volume of semen and serve a wider coverage of hens to be inseminated. In diluting semen for insemination, several factors need to be considered regarding the dilution effect on the quality of the sperm cells and the effect of reduced number of cells inseminated on fertility. A typical dilution effect was observed by Sexton (1976) when turkey semen was diluted at a ratio of 1:8 and 1:12. This dilution led to an increased respiration of cells and corresponding decline in fertilizing capacity. However, at dilution rates of up to 1:4 with Lake's diluents at weekly insemination intervals, no significant difference was observed in fertility with undiluted control when held at 25°C or 5°C for 30 minutes prior to insemination Sexton (1976). The objective of the study was therefore to assess the effect of the interaction of insemination intervals (one and two weeks) and semen dilution ratio (1:0, 1:1, 1:2, and 1:3, semen to diluent) on fertility, embryo mortality and hatch parameters in local turkey hens.

MATERIALS AND METHODS

The research was carried out at the poultry unit of the Teaching and Research Farm, University of Ibadan, Nigeria. Locally-adapted turkeys were used for the study, ten toms and sixty-four hens. 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.Factor A was the semen dilution ratio, while factor B

was the insemination intervals.The block effect was the hen age groups (old hens of 16 months old and young hens of 9 months old). Semen was harvested from the toms, pooled and 0.02mL inseminated into hens. A portion of pooled semen inseminated was evaluated for semen quality characteristics Hens in groups 1 to 4 were inseminated with semen diluted with normal saline at a ratio of 1:0, 1:1, 1:2 and 1:3 at weekly interval while those in groups 5 to 8 were inseminated at interval of 2 weeks with the same varied dilution ratios as with groups 1 to 4. Insemination and weekly egg collection for incubation was done for 12 weeks. Candling was done on day 25 of incubation. Fertility, embryo mortality, hatch of fertile eggs and hatch of set eggs were determined as described by Adebisi and Ewuola (2019b).Data collected were analysed using two-way analysis of variance (of square root transformed data) using general linear model of SAS (2003). Means were separated using Duncan's multiple range test of same software.

RESULTS

The characteristics of pooled semen prior to dilution and insemination over twelve weeks are presented in Table 1. Spermatozoa concentration ranged from $4.29x10^6$ – $6.12x10^6$ cells/mL, progressive spermatozoa motility, 80% - 95%, while liveability was 91.3% – 97.4%.

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

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

The effects of semen dilution ratio, insemination intervals and their interactions on fertility and hatch parameters are presented in Table 2. Results of the main effects indicated no significant difference in the parameters between weekly or fortnight insemination. However, fertility and hatch of set eggs in groups inseminated with a dilution ratio of 1:0 and 1:2 were significantly ($p<0.05$) higher than 1:3. Interaction of the factors also indicated a significantly ($p<0.05$) higher fertility in groups of hen inseminated with diluted semen ratio of 1:0 (89.3±2.8%) and 1:2 (86.8±3.0) than 1:3 (72.8±4.4%), but at 2 weeks insemination intervals only. These values were however, not significantly different from all other groups. There were no significant differences ($p>0.05$) in the hatch of fertile or set eggs. The block effect of hen age was also not significant for any of the parameters. The effect of the interaction of semen dilution ratio and insemination intervals on early (EEM), mid (MEM), late (LEM) and total (TEM) embryo mortality in turkeys is presented in Table 3. There were no significant ($p>0.05$) differences among the groups for each of the parameters.

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

Factors	Levels		Parameters measured (%)		
			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 ($p<0.05$)

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 along columns are not significant (P>0.05); EEM – Early Embryo Mortality; MEM – Mid Embryo Mortality; LEM – Late embryo Mortality; TEM – Total Embryo Mortality

DISCUSSION

The similarity observed for the weekly and fortnight insemination intervals is in line with observations from Adebisi and Ewuola (2019a) using undiluted semen for insemination in local turkeys. The significance of the interaction of the factors implied that at weekly intervals, fertility was similar across the dilution ratios, while at 2 weeks interval, 1:3 was significantly lower than 1:2 and undiluted semen. The mean characteristics of semen inseminated during the period of the study (Table 1) implied that 1:0, 1:1, 1:2 and 1:3 contained approximately $90x10^6$, $45x10^6$, $30x10^6$ and $22.5x10^6$ motile sperm cells, respectively per 0.02mL insemination dose. The results on 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, which is similar to 1:3 dilution at weekly intervals in this study. The significantly low fertility observed for the dilution ratio of 1:3 at 2 weeks interval may be as a result of an overall relatively lower number of sperm cells inseminated and length of insemination interval of two weeks. Ogasawara (1981) reported that at least 100 million sperm cells should be inseminated in turkeys to produce optimal fertility. Haque and Hossain (2011) suggested 80-100 million sperm cells, while Bramwell (2012) recommended 150 - 300 million viable sperm cells for insemination. 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. Requirements by Sexton (1976) may have similarities with the results from this study because the birds used at that time may not have had much genetic advancement, as with those used in this study, which were random-bred and unimproved strains.

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. They reported that in lines selected for rapid body growth, the dams affected fertility over time. The implication of this may mean more sperm cell 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 from large white turkeys when

inseminated with equal numbers of viable sperm cells (Christensen *et al.*, 2005). They therefore concluded that genetic selection for economically important traits may affect sperm hydrolysis of the perivitelline layer and subsequent fertility and embryo liveability. Despite the differences observed in fertility, the hatch parameters of the interaction of factors did not show significant differences. There were also no significant differences observed with the embryo mortality at various stages. 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 penetration of too few sperms into the perivitelline layer can be a consequence of very low number of inseminated sperm cells. The absence of significant differences in the embryo mortality and hatch parameters among the treatments is also an indication that the embryo mortality was not affected by the reduced number of sperm cells inseminated as implied from the higher dilution ratios of 1:2 and 1:3. They also added that when inseminated sperm cells are too low, female birds compensate by allowing a greater proportion of sperm reach the site of fertilisation to ensure sufficient supernumerary sperm cells penetrate the germinal disc.

CONCLUSION AND RECOMMENDATION

The result from this study therefore implied that as few as 22.5 million motile sperm cells (1:3 dilutions) inseminated at weekly intervals over a period of 12 weeks, was sufficient to ensure fertilisation and embryo survival. However, if fortnight insemination is to be adopted, semen for insemination should not be diluted beyond 1:2 (a minimum of $30x10^6$ motile spermatozoa).

REFERENCES

Adebisi K. A and Ewuola E. O. 2019a. Egg fertility and embryo viability of turkey hens inseminated at varied intervals. *LRRD. 31 (6), article # 87.*

Adebisi K. A. and Ewuola E. O. 2019b. Comparative effects of saline solutions as diluents on *in-vitro* semen storage, egg fertility and hatchability in turkey hens. *Nigerian J. Anim. Sci.* 2019, 21(3): 107-116.

Bramwell, R.K., 2012. An overview of artificial insemination in poultry. The Merck veterinary manual for veterinary professionals. Retrieved Sept. 11, 2014 from www.merckmanuals.com.

Hemmings, N. and Birkhead, T. R. 2015. Polyspermy in birds; sperm number and embryo survival. Proccedings of the royal society of biological sciences. Published 28th October, 2015. Doi:10.1098/rspb.2015.1682.

Christensen, V.L., Fairchild, B. D., Ort, D. T. and Nestor, K. E. 2005. Dam and sire effects on the periviteline Layer and resulting fecundity of different lines of turkeys. *JAPR* 14: 483-491.

Haque, N. and Hossain, S.A. 2011. Methods of mating and artificial insemination in poultry. Retrieved Sept. 18, 2014 from http://www.poulvet.com

Ogasawara, F.X. 1981. Mechanisms associated with reproductive tissues and their implications on fertility level and duration: Reproductive efficiency of turkeys. *Western Regional Research Publication*. 142: 26 - 44.

Sexton, T. J. 1976. Studies on the dilution of turkey semen. *Br. Poult. Sci.* 17(2):179-184.