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Sperm competition and fertilization in Turkeys

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Abstract

Tandem artificial inseminations using semen from Large White and Broad Breasted Bronze turkey males were preformed on Large White hens. The percentage of phenotypes which hatched from stored and newly inseminated spermatozoa were assessed to determine the competitive fertilization between stored and newly inseminated cells. Treatments utilizing two different insemination doses (50 or 200 million spermatozoa) inseminated at two intervals (weekly or biweekly) were used in conjunction with the tandem matings to determine the relationship between fertility and spermatozoal interactions. Highest fertility was observed when 200 million spermatozoa were inseminated weekly whereas the poorest fertility resulted when 50 million spermatozoa were inseminated biweekly. In the high fertility group, most of the fertility resulted from newly inseminated spermatozoa. Conversely, in the low fertility group, more fertility occurred from stored spermatozoa. It is concluded that the oviduct may function to affect fertility differently depending upon the competition between stored and newly inseminated spermatozoa.

Introduction

Competitive interactions of spermatozoa and the oviduct are only partially understood. In avian species which store spermatozoa for extended periods of time the interaction becomes complicated by the presence of spermatozoa which are already residing in oviducal storage sites. The relationship between these oviducal-spermatozoal interactions and fertility is also poorly understood. Studies to understand the sperm-oviduct relationship in chickens were undertaken a number of years ago (Bobr et al., 1964) and subsequent studies (Burke et al., 1969; Compton and Van Krey, 1979a; Compton and Van Krey, 1979b) have suggested the existence of various relationships between stored spermatozoa and subsequent fertility.

Only about 5% of the turkey spermatozoa in an insemination dose of 150 million spermatozoa will eventually reside in sperm storage sites (Bakst, 1989). Better insemination technology may help improve this retention rate. Sexton (1977) suggested that low fertility during the early stages of production in turkeys could be

attributed to inadequate sperm numbers residing in storage sites and suggested an insemination dose of 200 million viable spermatozoa per week per hen. Improved fertility has also been observed in turkey hens when inseminations were performed just prior to the onset of lay when compared to hens inseminated after the onset of egg production (McIntyre et al., 1982). They suggested that the increased fertility may indicate a vital role for the spermatozoa placed into the oviduct during the initial inseminations because the differences in fertility seen between early and late insemination groups were evidenced by differing fertility rates 7 to 8 weeks later. This suggests a competitive nature for stored and newly inseminated spermatozoa in the turkey hen oviduct. The competition for fertilization between stored spermatozoa and spermatozoa added by subsequent inseminations was the subject of the present study. The objective of the present study was to observe the fertility and disappearance of spermatozoa inseminated before the initial oviposition when spermatozoa of another phenotype were used in subsequent insemination treatments. Understanding spermatozoal competition can help improve the economics of fertility of avian species.

Materials and Methods

Large White turkey hens (Hybrid Turkeys, Inc., Kitchener, Ontario, Canada) were hatched in June and grown to sexual maturity (30 weeks) on the University research farm under standard management conditions described elsewhere (Christensen and Bagley, 1989). The hens were photostimulated (15.5 hr L and 8.5 hr D; lights on 5:00 to 20:30) when moved to the laying house at 30 wks of age. On days 14 and 17 following photostimulation, the hens were each inseminated with 200 million viable spermatozoa using semen from Broad-Breasted Bronze or Large White males which were hatchmates of the hens. Half of each insemination (AI) was inseminated with Large White semen and half was inseminated with semen from Broad-Breasted Bronze males. After the initial AI, half of each treatment was inseminated with the opposite phenotype for the remainder of the experiment. Poult phenotype was then assessed as a factor in a factorial arrangement of the experiment. The percentage of phenotypes due to the initial AI was computed by dividing the number of identifiable phenotypes due to the initial AI by the total number of phenotypes observed from both sire phenotypes. The technique has been described in greater detail elsewhere (Christensen, 1981; Christensen and Bagley, 1989).

The AI treatments imposed upon the hens following the initial AI were as follows: 1) 24 hens were inseminated with 50 million viable spermatozoa at weekly (50 WK) or biweekly (50 BWK) intervals, and 2) 24 hens were inseminated with 200 million viable spermatozoa at weekly (200 WK) or biweekly (200 BWK) intervals. The experimental AI were performed for 20 weeks following the initial AI. Spermatozoa viability was

determined prior to AI by the fluorometric technique of Bilgili and Renden (1984). Dilutions were made using Minnesota Turkey Growers Association diluent (Minnesota Turkey Growers Association, St. Paul, MN 55114) to insure inseminating the appropriate viable spermatozoa numbers in .033 ml/AI.

Eggs were collected from the laying house five times daily and stored at 12.8°C and 75% relative humidity before setting in incubators as weekly intervals. Weekly fertility was determined at hatching for 20 weeks of lay for each treatment. All eggs failing to hatch were broken, and the contents were examined to determine true fertility and approximate embryonic age at death. Fertility was calculated beginning the 2nd day following the initial AI. At the time of hatching, phenotypes due to each treatment were recorded as well as identifiable phenotypes in nonhatching eggs.

The 20-week laying cycle of the hens was divided into four periods of 5 weeks each for statistical analysis. The data were arranged in a factorial design for analysis within each time period. The factors in the design were phenotype order (Bronze or White), AI dose (50 or 100 million spermatozoa) and AI interval (weekly or biweekly). The analysis was performed by the general linear models procedure and all possible interactions were tested. Trend analyses were performed to aid in interpreting significant interactions.

Results

Phenotype order displayed significant fertility effects; Bronze males fertilized more eggs than Large White males. In no case was there a significant phenotype order x treatment interaction, suggesting that variation from phenotype order did not affect the treatments. Fertility during weeks 1 to 5 and weeks 6 to 10 of egg production (Figure 1) was depressed ($P \leq .05$) by biweekly AI intervals compared to weekly and by the 50 million spermatozoa AI dose ($P \leq .05$) compared to the 200 million spermatozoa dose. The interaction of the main effects of AI interval and AI dose was not significantly affected. Conversely, during weeks 11 to 15 and 16 to 20 of egg production, a significant ($P \leq .05$) interaction of AI interval and dose was observed. The combined treatments of biweekly AI interval and 50 million spermatozoa AI dose have additive effects in depressing fertility whereas biweekly AI intervals and 200 million spermatozoa AI dose did not.

The percentages of phenotypes at hatching resulting from the stored spermatozoa in each treatment are given in Figure 2. Significant ($P \leq .05$) differences between treatments occurred during weeks 1 to 5 due to both AI intervals and AI doses. During weeks 6 to 10 significant differences due to AI interval occurred, but no differences in phenotype percentages were observed between AI doses.

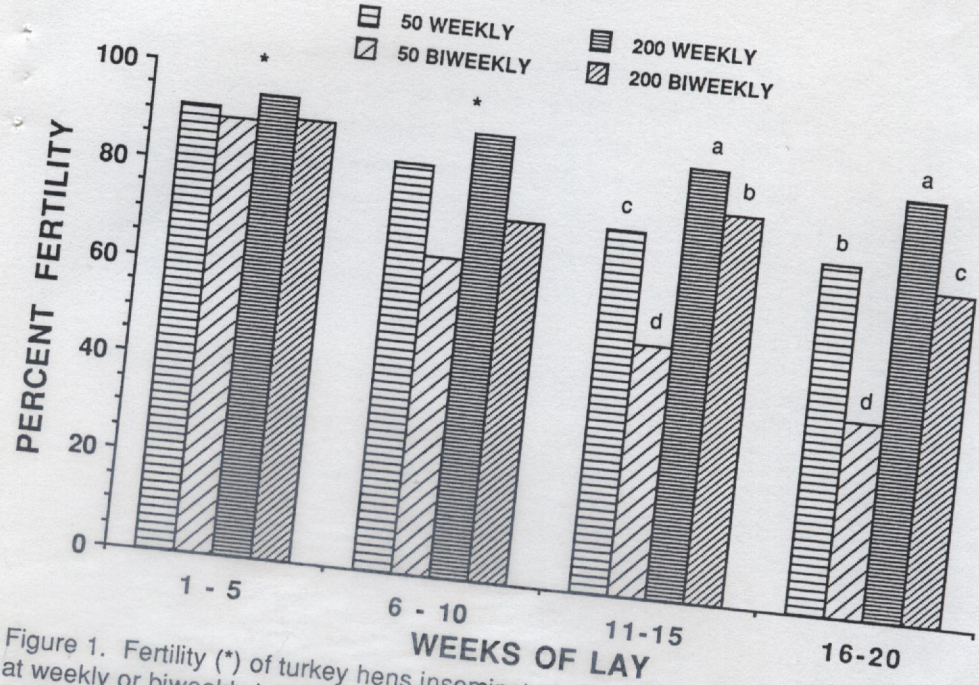

Figure 1. Fertility (*) of turkey hens inseminated with 50 or 200 million spermatozoa at weekly or biweekly intervals. Star (*) denotes differences between main effects. Small case letters (a, b, c, d) denote a significant interaction.

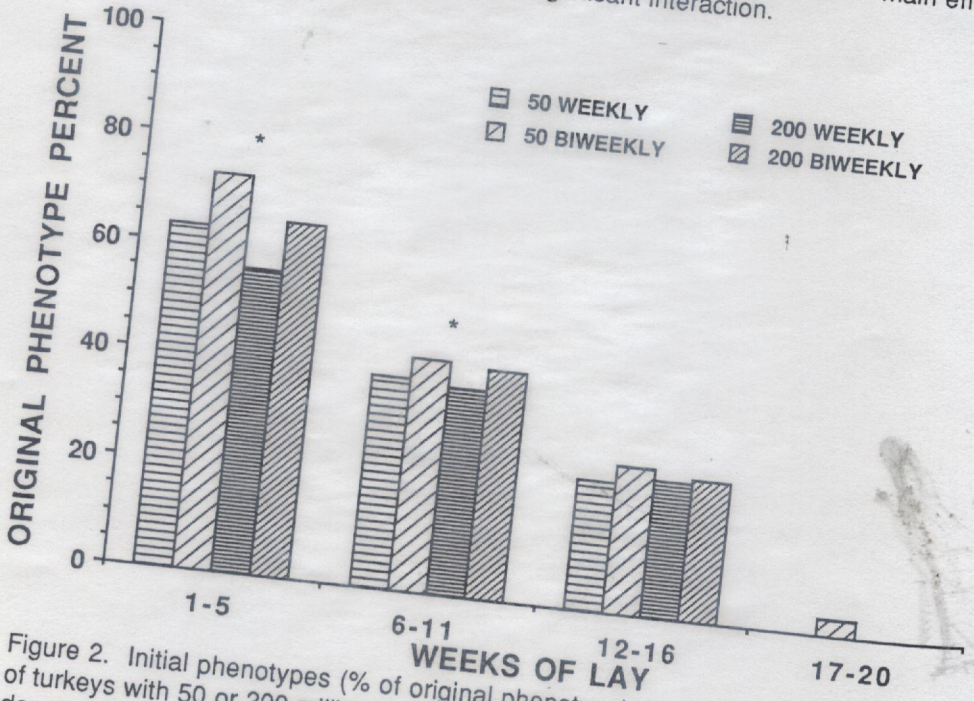

Figure 2. Initial phenotypes (% of original phenotype) identified following insemination of turkeys with 50 or 200 million spermatozoa at weekly or biweekly intervals. Star (*) denotes differences ($P < 0.05$) between main effects.

Trend analysis of both fertility and the percentage of phenotype data regressed over time indicated significant ($P \leq .0001$) linear effects of all AI treatments. The slope of the regression line for the 200 week treatment differed from the slope of the 50 BWK treatment line for both fertility ($P \leq .0001$) and percentage phenotypes ($P \leq .05$) (Table 1). The 200 week percentage phenotypes declined more rapidly than did the 50 BWK and had a slower decline in fertility over time. No differences were observed between the 200 BWK and 50 WK AI treatments for fertility or percentage phenotypes.

TABLE 1. Regression lines for fertility and percent of initial phenotypes regressed on time of lay (n=6)

Treatment[1]	Regression Equation
	Fertility
BK 200	$Y = -.061X^{a} + .931$
WK 200	$Y = -.028X^{b} + .969$
BK 50	$Y = -.160X^{a} + 1.015$
WK 50	$Y = -.067X^{a} + .969$
	Percent Phenotype
BK 200	$Y = -.087X^{ab} + .556$
WK 200	$Y = -.058X^{a} + .447$
BK 50	$Y = -.103X^{b} + .628$
WK 50	$Y = -.087X^{ab} + .531$

[1] BK = biweekly insemination interval; WK = weekly insemination interval; 50 = 50 million viable spermatozoa per hen; 200 = 200 million viable spermatozoa per hen.

[a,b] Slopes followed by a different superscript differ significantly ($P \leq .0001$).

Discussion

The fertility observed in the present study following weekly inseminations with 200 million viable spermatozoa at weekly intervals was superior to that of all other treatment combinations. This observation is in agreement with the results in prior studies (Sexton, 1977; Christensen and Bagley, 1989). Inseminating 50 million viable

spermatozoa at either a weekly or biweekly interval did not support acceptable levels of fertility for most of the experiment. Because all treatments were identical insofar as the initial inseminations were concerned, implications of these observations are due to the competition between stored spermatozoa already residing in the oviduct and spermatozoa subsequently inseminated.

It is assumed that the two AI performed before the initial oviposition filled the spermatozoa storage sites to capacity because under similar conditions a single AI in the absence of oviposition filled storage sites to capacity 24 hours following the AI (McIntyre and Christensen, 1983). Replacement of stored spermatozoa from the initial AI occurred differently depending upon treatments. The treatment combination resulting in the best fertility (200 million spermatozoa inseminated weekly) relied least on stored spermatozoa for fertilization whereas the treatment combination resulting in the lowest level of fertility (50 million spermatozoa inseminated biweekly) relied the most on stored spermatozoa for fertilization. Phenotypes from the 50 million biweekly treatment were observed an average of 15.3 ± 1.3 weeks of egg production whereas they were seen an average of 13.3 ± 1.1 weeks of egg production in the 50 million weekly treatment. This suggests that when spermatozoa numbers in the oviduct become marginal (50 million cells), the oviduct can utilize stored viable spermatozoa for an extended period of time to maintain some level of fertility. The stored spermatozoa competed better for fertilization than did the recently inseminated spermatozoa when 50 million spermatozoa were inseminated at biweekly intervals. This improved competition was not observed when 200 million spermatozoa were inseminated biweekly or when 50 million cells were inseminated at a weekly interval. This suggests also that the turkey hen oviduct may be able to maintain viable spermatozoa for longer periods than previously observed (Lorenz, 1966) when the proper physiological conditions exist.

The length of time the hens had been laying eggs influenced the competition of the phenotype spermatozoa. Inseminating 200 million spermatozoa weekly resulted in fertility superior to all other treatments at all time periods and inseminating 50 million spermatozoa biweekly resulted in the poorest fertility at all time periods. The interaction occurred when the fertility plateaued in the final two periods due to AI with 50 million spermatozoa inseminated weekly, whereas fertility resulted from inseminating 200 million spermatozoa biweekly actually increased during weeks 11-15 of lay. These changes in fertility occurred with no differences in the percentage of phenotypes from the original inseminations. Therefore, the spermatozoa from the original inseminations are not competing differently when 50 or 200 million cells are inseminated at weekly or biweekly intervals. According to Bakst (1989) the turkey has, before the onset of egg production, the biological potential to store spermatozoa in its oviduct up to 5 weeks, yet after the onset of egg production fewer spermatozoa are retained by the oviduct. Thus, a possible explanation of the observations may be

that the spermatozoa from the original phenotype are depleted and 50 million spermatozoa inseminated subsequently weekly maintained fertility at an unacceptable low rate. However, when presented with a 4-fold greater number of viable spermatozoa, the oviduct was able to sustain higher levels of fertility presumably because the larger number of spermatozoa was able to replace the stored spermatozoa more effectively.

To better understand the interactions occurring during the latter times of lay the rate of decline of both fertility and percentage of phenotypes was tested by regressing both variables by treatments on time of lay. The slopes of the resulting regression lines were tested for significance by analysis of variance. As might be expected, fertility declined slower and the percentage phenotype declined faster in the treatment inseminated with 200 million spermatozoa at weekly intervals than the 50 million cells inseminated at biweekly intervals. This follows because of the greater number of cells inseminated; however, the surprising result occurred between inseminating 200 million spermatozoa biweekly and inseminating 50 million spermatozoa weekly. The 200 BWK and 50 WK treatments did not differ in rate of decline in fertility or percentage phenotypes. This suggests an equivalent fertility and spermatozoa utilization may occur with a 50% reduction in the number of spermatozoa inseminated. This may aid in maximizing the utilization of gametes from superior sires (Sexton, 1983).

In conclusion, the competition between spermatozoa stored in the oviduct and newly inseminated spermatozoa may affect subsequent fertility. The competition may more effectively utilize spermatozoa from superior sires in commercial poultry operations. More data are needed to more clearly understand the competition for storage sites in avian species to improve fertility of avian species.

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INSEMINATIONS COMPETITIVES ET FERTILISATION CHEZ LA DINDE

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Résumé

Nous avons utilisé des inséminations en tandem pour féconder des dindes Large White avec du sperme issu de mâles Large White et Broad Breasted Bronze. Les pourcentages de phénotypes de poussins issus de sperme conservé ou au contraire fraîchement inséminé ont été calculés afin de mesurer le degré de fertilisation compétitive dû aux spermatozoïdes conservés ou récemment inséminés. Deux doses différentes d'insémination (50 et 200 millions de spermatozoïdes) ainsi que deux fréquences d'insémination (1 fois/sem ou 1 fois/2 sem) ont été utilisées en conjonction avec les inséminations en tandem pour déterminer la relation entre la fertilité et les intéractions entre spermatozoïdes. La plus haute fertilité a été obtenue à partir d'inséminations hebdomadaires de 200 millions de spermatozoïdes et la plus faible à partir d'inséminations à 2 semaines d'intervalle de 50 millions de spermatozoïdes. Dans le groupe à haute fertilité, la plupart des descendants étaient issus du dernier sperme mis en place (le plus récent). Par contre, dans le groupe à basse fertilité, une partie importante des descendants était issue de la première insémination. En conclusion, l'oviducte peut fonctionner différemment selon le degré de compétition existant entre les spermes mis en place en première ou en deuxième insémination.

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