Decreased sperm survivability in subfertile Delaware roosters as indicated by comparative and competitive fertilization

J. D. Kirby, D. P. Froman, H. N. Engel, Jr* and P. E. Bernier

Department of Poultry Science, *College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331-3402, USA

Summary. Duration of fertility following intravaginal and intramaginal insemination of hens with viable spermatozoa from subfertile Delaware roosters was compared with that obtained with spermatozoa from fertile Leghorns and subfertile Wyandotte roosters. In contrast to results with Leghorn and Wyandotte birds, duration of fertility was not increased following intramaginal insemination of spermatozoa from Delaware birds. Competitive fertilization also demonstrated that duration of fertility was less than expected in the spermatozoa from Delaware birds. Heritable subfertility in Wyandotte and Delaware roosters therefore appears to be attributable to distinct sperm defects.

Keywords: spermatozoa; chicken; fertility; sperm competition

Introduction

Froman & Bernier (1987) identified a heritable reproductive disorder in roosters within a line of Delaware chickens maintained by the Oregon Agricultural Experiment Station. This disorder was characterized by sperm degeneration within the mid- and distal ductus deferens which appeared to account for the subfertility that originally drew attention to these roosters. Because seminal quality was improved by changing the frequency of ejaculation from a weekly to a daily basis, the extent of sperm degeneration appeared to depend upon the duration of sperm residence within the ductus deferens. Therefore, Froman & Bernier (1987) suggested that a dysfunction of the ductus deferens resulted in sperm degeneration therein.

However, this hypothesis is tenable only if a sperm defect can be discounted. After spermiogenesis, chicken spermatozoa are maintained within the distal ductus deferens for several days before being ejaculated or voided into the cloaca (de Reviers, 1975). After ejaculation, chicken spermatozoa can be maintained within the hen's uterovaginal sperm storage glands for many days before being made available for fertilization (Bohr *et al.* 1964). Consequently, a diminished duration of fertility would be symptomatic of a sperm defect. Therefore, the objective of the present work was to assess the duration of fertility of viable spermatozoa ejaculated from roosters characterized by the reproductive disorder described by Froman & Bernier (1987).

Materials and Methods

Experiment 1

Semen was collected from roosters representing 4 genotypes: Single Comb White Leghorn (SCWL; N = 3), Silver-Laced Wyandotte heterozygous for rose comb (N = 4), subfertile Delaware (N = 5), and Silver-Laced Wyandotte homozygous for rose comb (N = 5). All roosters had been ejaculated weekly for ≥ 3 weeks before this experiment.

With the exception of the Delaware birds, roosters were ejaculated weekly throughout the experiment. Daily ejaculation of Delaware cocks began 4 days before the first insemination and continued every other day thereafter.

Semen was pooled according to rooster genotype. Percentages of viable spermatozoa as well as sperm concentrations were determined fluorometrically by ethidium bromide uptake (Bilgili & Renden, 1984). Each of the 4 semen samples was diluted to 2.0×10^9 viable spermatozoa/ml with Beltsville Poultry Semen Extender, pH 7.5 (BPSE; a gift from Dr T Sexton, USDA, Beltsville, MD, USA). Each sperm suspension was used to intravaginally inseminate 30 SCWL hens with 1×10^8 viable spermatozoa per hen. Three replicate fertility trials were completed for each rooster genotype.

Egg collection began on the 2nd day after insemination and continued for 21 days. Eggs were set weekly. Fertility was assessed by breaking eggs open after 4 days of incubation and examining the contents for embryonic development. Arc sine transformations of percentages of fertilized eggs were analysed by single classification analysis of variance (Sokal & Rohlf, 1981)

Data were pooled when no difference ($P \geq 0.05$) was observed among replicate fertility trials. Percentages of fertilized eggs were plotted by day. Because plots conformed to logistic functions, parameters of

$$y(x) = \frac{\gamma}{1 + e^{B(x-\tau)}}$$

were estimated by iterative least squares (Freund & Littell, 1986a)

Experiment 2

Semen was collected and sperm suspensions prepared as above with two exceptions. First, semen from Wyandotte roosters heterozygous for rose comb was not used. Second, each sperm suspension contained 5×10^8 viable spermatozoa/ml. For each sperm suspension, each of 25–30 SCWL hens was inseminated intramagnally with 5×10^7 viable spermatozoa via laparotomy (Howarth, 1983). Egg collection, incubation, and data analyses were performed as above.

Experiment 3

Semen was collected from the Delaware roosters used in the preceding experiments as well as from 5 Brown Leghorns. The latter had been characterized as having a fertilizing ability equivalent to that of the SCWL roosters ($P > 0.05$). Ejaculates were pooled according to breed. After the seminal evaluations described above, the semen samples from the Delaware and Brown Leghorn birds were diluted with BPSE to 2.0×10^9 viable spermatozoa/ml. Equal volumes of these sperm suspensions were mixed, and each of 40 New Hampshire hens was inseminated intravaginally with 1×10^8 viable spermatozoa from this mixture.

Eggs were collected and set as above. However, eggs were incubated for 21 days and down colour of chicks was assessed at hatch. Down colour was also recorded for feathered chicks that failed to hatch. Percentages of chicks sired by Delaware birds were plotted by day. Parameters of

$$y(x) = \alpha + \beta_1 x + \beta_2 x^2$$

were estimated by weighted least squares (Freund & Littell, 1986b).

Experiment 4

Semen was collected and sperm suspensions prepared according to the preceding experiment with one exception: each sperm suspension contained 5×10^8 viable spermatozoa/ml. Each of the 40 New Hampshire hens used in the preceding experiment was inseminated intramagnally (Howarth, 1983) with 5×10^7 viable spermatozoa. Egg collection, incubation, and chick down colour were determined as above. Parameters of

$$y(x) = \alpha + \beta x$$

were estimated by least squares (Freund & Littell, 1986a).

Results

As shown in Table 1, daily ejaculation of subfertile Delaware roosters for 5 consecutive days and every other day thereafter yielded percentages of viable spermatozoa that were comparable to those of Leghorn and Wyandotte birds. However, as shown in Table 2, sperm fertilizing ability was not equivalent among rooster genotypes. The means in Table 2 represent those of pooled data as no differences were observed ($P > 0.05$) among replicate fertility trials. The fertilizing ability of spermatozoa from Delaware birds was intermediate ($P < 0.001$) between that of Wyandottes homozygous for rose comb, which are known to be characterized by subfertility (Crawford & Merritt, 1963), and that of Wyandottes heterozygous for rose comb, which like Leghorns, are not subfertile.

Table 1. Percentages of viable spermatozoa ejaculated by 4 genotypes of rooster

Breed of rooster	No. of birds	Comb genotype	Sperm viability (%)
White Leghorn	3	rrpp	100 $\pm$ 0.0
Wyandotte	4	Rrpp	100 $\pm$ 0.0
Delaware	5	rrpp	98 $\pm$ 1.1
Wyandotte	5	RRpp	96 $\pm$ 1.0

Values represent the mean $\pm$ s.e.m. for 3 replicates of pooled semen.

Table 2. Fertility of Single Comb White Leghorn hens over a 21-day egg collection interval following a single intravaginal or intramaginal insemination with 1×10^8 and 5×10^7 viable spermatozoa, respectively

Insemination route	Breed of semen donors	Comb genotype	No. of eggs	Fertility (%)
Intravaginal	White Leghorn	rrpp	1366	51 $\pm$ 1.2*
	Wyandotte	Rrpp	1360	47 $\pm$ 1.4*
	Delaware	rrpp	1295	42 $\pm$ 1.3
	Wyandotte	RRpp	1348	26 $\pm$ 1.4*
Intramaginal	White Leghorn	rrpp	161	64 $\pm$ 1.2*
	Delaware	rrpp	202	41 $\pm$ 0.4
	Wyandotte	RRpppp	176	57 $\pm$ 1.0*

Fertility values are mean $\pm$ s.e.m.

* $P < 0.001$ compared with that for Delaware roosters.

Fertility after intramaginal insemination of spermatozoa from Leghorn, Delaware or subfertile Wyandotte roosters is shown in Table 2. When compared to values obtained with intravaginal insemination, fertility for the Leghorn and subfertile Wyandotte birds increased by 13 and 30%, respectively, but there was no change for Delaware roosters.

These relationships were more evident after graphical analyses (Fig. 1). Estimates of τ , time at which half-maximal fertility occurred, were 11.8, 9.1 and 6.0 days after intravaginal insemination for Leghorn, Delaware and subfertile Wyandotte birds, respectively, and 15.2, 9.6 and 13.9 days after intramaginal insemination. For spermatozoa from Delaware cocks, the hypothesis that 9.1 and 9.6 were estimates of the same unknown parameter was not rejected ($P > 0.05$). Thus, unlike the spermatozoa from the Leghorn and subfertile Wyandotte birds, intramaginal insemination of those from Delaware birds failed to increase duration of fertility.

When New Hampshire hens were inseminated intravaginally or intramagnally with spermatozoa from Delaware and Brown Leghorn roosters, most chicks were sired by Brown Leghorns (Table 3). As shown by the fitted curves in Fig. 2, percentages of chicks sired by Delaware birds were not constant throughout egg collection intervals. The hatch of fertile eggs after intramaginal insemination (Table 3) also shows that the insemination dose of 5×10^7 spermatozoa did not increase the incidence of embryonic mortality.

Discussion

Heritable subfertility in roosters has been reported in two instances: roosters homozygous for rose comb (RR; Crawford & Merritt, 1963) and Delaware roosters (Froman & Bernier, 1987). The

Fig. 1. Duration of fertility after intravaginal (●) and intramaginal (△) insemination of Single Comb White Leghorn hens with spermatozoa from (a) Single Comb White Leghorn roosters, (b) Delaware roosters, and (c) Wyandotte roosters homozygous for rose comb. Insemination doses were 1×10^8 and 5×10^7 viable spermatozoa, respectively. Solid lines represent the functions

$$\begin{aligned} \text{(a) } y(x) &= \frac{97}{1 + e^{-0.5340(11.8-x)}} \text{ and } y(x) = \frac{94}{1 + e^{-0.7634(15.2-x)}}, \\ \text{(b) } y(x) &= \frac{100}{1 + e^{-0.4266(9.1-x)}} \text{ and } y(x) = \frac{100}{1 + e^{-0.7507(9.6-x)}}, \\ \text{and (c) } y(x) &= \frac{94}{1 + e^{-0.4653(6.0-x)}} \text{ and } y(x) = \frac{97}{1 + e^{-0.8782(13.9-x)}}. \end{aligned}$$

latter are characterized by a single comb and a Columbian feather colour pattern. In each case, subfertility has been attributed to reduced sperm fertilizing ability. However, the mechanisms responsible for subfertility appear to be distinct.

Buckland *et al.* (1969) attributed subfertility in RR roosters to a reduced sperm fumarase activity and suggested that this difference might affect sperm survivability within the oviduct. In view of the duration of fertility observed following the intramaginal insemination of RR Wyandotte spermatozoa (Fig. 1c) as well as the inability of Petitjean & Servouse (1981) to detect reduced fumarase activity in extracts of spermatozoa from RR roosters, the hypothesis of Buckland *et al.* (1969) is untenable. Petitjean & Servouse (1981) did, however, report that spermatozoa from RR roosters were characterized by poor motility. Thus, in agreement with Etches *et al.* (1974),

Table 3. Comparison of fertility, hatchability, and paternity of chicks from eggs laid by New Hampshire hens over a 2-week interval following insemination with spermatozoa from Delaware and Brown Leghorn roosters

Type of insemination	No. of eggs	Fertility (%)	Hatch of fertilized eggs (%)	Chicks sired by Delawares (%)
Intravaginal	407	69	71	36
Intramaginal	365	70	75	33

Insemination doses were 1×10^8 and 5×10^7 viable spermatozoa per hen for intravaginal and intramaginal inseminations, respectively. In either case, 50% of the spermatozoa inseminated were from Delaware roosters.

Fig. 2. Percentages of chicks sired by Delaware roosters (▲) following (a) intravaginal and (b) intramaginal insemination of New Hampshire hens with equal numbers of spermatozoa from Delaware and Brown Leghorn roosters. In (a) the solid line ($R^2 = 0.87$) represents the function

$$y(x) = 37.5 + 1.01(x) - 0.214(x^2),$$

and the datum from Day 11 was excluded from the estimation of the fitted curve as a consequence of outlier detection (Freund & Littell, 1986c). In (b) the solid line ($R^2 = 0.94$) represents the function

$$y(x) = 68.9 - 5.6(x).$$

the subfertility of RR Wyandotte birds after intravaginal insemination (Fig. 1c) may be attributable to inadequate sequestration of spermatozoa within the uterovaginal sperm storage glands.

In contrast, Froman & Bernier (1987) concluded that sperm degeneration accounted for subfertility of Delaware roosters. Theoretically, sperm degeneration within the ductus deferens could be the consequence of an autoimmune condition, a dysfunction of the ductus deferens, or a sperm defect. The autoimmunity hypothesis has been tested and rejected (unpublished data). Of the two remaining hypotheses, sperm autolysis warranted investigation first because such a phenomenon, were it to occur, would confound any investigation of the sperm maintenance function of the ductus deferens. We proposed that, if an aberrant duration of fertility were

observed following the insemination of viable Delaware spermatozoa, then a sperm defect would be likely.

In Exp. 1, the fertilizing ability of spermatozoa from Delaware roosters was compared with those of a genotype characterized by subfertility and two genotypes that are not. As shown in Table 2, the fertilizing ability of Delaware birds was intermediate ($P < 0.001$) between that of the RR and Rr Wyandottes. Even so, the fertilizing ability of Delaware chicken spermatozoa after intravaginal insemination was more comparable to that of the Rr Wyandotte as well as New Hampshire and Barred Plymouth Rock birds (unpublished data) than it was to that of RR Wyandotte roosters. The implicit ranking of fertilizing abilities in Table 2 is not only typical among breeds but also among individuals within breeds (Allen & Champion, 1955; Martin & Dziuk, 1977). The fertilizing ability of spermatozoa from Delaware roosters in Exp. 1 was more typical of spermatozoa from American breeds than that of subfertile roosters *per se*.

However, when intramaginal inseminations were performed, spermatozoa from Delaware birds failed to yield fertility greater than that observed following intravaginal insemination. As was expected based upon the work of Van Krey *et al.* (1966), intramaginal insemination with spermatozoa of SCWL birds increased fertility (Fig. 1a). This was also true with spermatozoa from RR Wyandottes (Fig. 1c). The latter observation may be explicable in terms of circumventing sperm sequestration within the uterovaginal sperm storage glands; Howarth (1983) demonstrated that intramaginal insemination of testicular spermatozoa, which are characterized by poor motility and an inability to fertilize ova following intravaginal insemination, yielded fertility equivalent to that obtained with ejaculated spermatozoa similarly inseminated. Because the two estimates of τ appeared to be independent estimates of the same unknown parameter only in the case of Delaware spermatozoa, we concluded that intramaginal insemination of spermatozoa from Delaware birds revealed an aberrant duration of fertility.

Competitive fertilization was performed to characterize further the fertilizing ability of viable spermatozoa from affected Delaware roosters. According to Martin *et al.* (1974), the insemination of hens with equal numbers of spermatozoa from Leghorns and Columbian roosters yielded disproportionate numbers of offspring, but the proportions were constant over a 15-day egg collection interval. We anticipated a declining proportion of chicks sired by Delaware roosters under similar conditions. In these experiments, Brown Leghorns were used rather than White Leghorns because the former sire a brown chick when crossed with a New Hampshire hen whereas Delawares and White Leghorns sire yellow chicks. Preliminary work had shown that the fertilizing ability of spermatozoa of Brown Leghorns was equivalent to that of spermatozoa from White Leghorns ($P > 0.05$). More importantly, preliminary work had also shown that there was no difference in the percentage of embryonic dead when New Hampshire hens were inseminated with spermatozoa from Brown Leghorn or Delaware cocks ($P > 0.05$). As expected, the proportion of chicks sired by Delaware roosters declined as a function of time following intravaginal and intramaginal insemination (Fig. 2). However, the high percentage of chicks sired by Delaware roosters over the first 3 days after intramaginal insemination was not anticipated.

Froman & Bernier (1987) observed that once the percentage of degenerate spermatozoa in ejaculates from subfertile Delaware roosters had been reduced to $\leq 5\%$ via frequent ejaculation, the amount of sperm degeneration increased after 3 and was maximal after 12 days of sexual rest. In the present work, daily fertility following the intramaginal insemination of spermatozoa from Delaware birds decreased from 100 to 14% over a 12-day interval. In contrast, when equal numbers of spermatozoa from SCWL and RR Wyandotte birds were inseminated similarly, daily fertility was $\geq 80\%$ over the same interval. It therefore appears that heritable sperm degeneration within the ductus deferens of the chicken may be attributable to a sperm defect. A premature activation of sperm metabolism might account for sperm degeneration within the ductus deferens and the transient initial superiority of spermatozoa from Delaware roosters.

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