Effects of Diethylstilbestrol Administration on Sperm Motility and Reproductive Function in Male Japanese Quail (Coturnix japonica)

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The present study was undertaken to evaluating the effects of diethylstilbestrol (DES) on sperm motility and reproductive function in male Japanese quail. To do so, we have identified the method that ensures maximal quail sperm motility, i.e. the semen from the ductus deferens was diluted with Lake's solution supplemented with 0.05% (w/v) caffeine and 10% (v/v) quail cloaca fluid. Sperm motility was analyzed by the computer-assisted sperm motility analysis system (CASA). Compared with control birds, administration of DES (0.1 or 1 mg DES on a daily basis for a week) caused a significant reduction in testicular weight, a marked disintegration of the seminiferous epithelium and a significant decrease in the sperm population of the seminiferous lumen. Sperm recovery and motility also showed a significant decrease in treated individuals. These results suggested that enhanced estrogenic effect caused by DES injection could cause a disruption in reproductive function by affecting spermatogenesis and influencing sperm motility.

Key words: diethylstilbestrol, male reproductive function, quail, sperm motility

Introduction

During the quail reproductive season, sperm are constantly being formed in the testis, followed by their maturation in the epididymis and ductus deferens. Furthermore, males have the ability to copulate with females and ejaculate sperm from the papilla of ductus deferens into the female cloaca with fluid from the accessory genital glands. Thus mated, females continue to produce fertilized eggs for a certain period.

Recently, it has been reported that endocrine disrupters might cause the reproductive abnormalities in a number of animal species (Khan et al., 1998; Atanassova et al., 1999; Stoker et al., 2000). In birds, exposure of embryos to estrogenic compounds causes abnormal formation of sexual organs (Perrin et al., 1995; Berg et al., 1999; Masuda and Koyanagi, 2001). However, little is known about the endpoint of endocrine disruptor action in adults. A significant goal in the studies of endocrine disruptor is to find the endpoint of their effects. It is assumed that majority of endocrine disruptors exert estrogenic effects, and may cause reproductive disorder.
In domestic animals such as bulls, pigs and chickens, many studies into artificial insemination have been performed. Methods for semen collection and treatment are well established and have contributed towards the development of artificial insemination techniques in these animals. In many cases, the motility, density and abnormality of sperm have been used to evaluate male fertility. However, there are even fewer studies on the collection and treatment of semen and their effects on sperm viability in quail.

The goal of present study was to evaluate an estrogenic endocrine disruptor on reproductive function in quail and to establish the endpoint effect. However, in order to perform the study, we needed to establish the quality of the sperm affected by the endocrine disruptor by evaluate sperm motility. Diethylstilbestrol (DES), which is an estrogenic compound, was used as a model chemical of endocrine disruptor.

**Materials and Methods**

**Experiment 1:** Methods for sperm collection, the choice of a suitable diluent as well as the assessment of sperm motility using a computer-assisted sperm motility analysis system (CASA) were the primary objectives of this experiment, i.e. we attempted to collect semen from cloaca by abdominal massage method (Bogdonoff and Shaffner, 1954) and from the nipple of the ductus deferens after decapitation to perform the sperm motility assay using the CASA system. Then, we examined the addition of caffeine and cloaca fluid to a diluent to increase sperm motility and reduce sperm clumping.

**Experiment 2:** The effects of DES administration on male quail reproductive function (the effects on the testicular weight, histology of the testis and sperm motility) were examined in experiment 2. Testicular weight was corrected for every 100 g in body weight (BW).

Japanese quail of 7-8 weeks old were used throughout this study. They were kept under a 14 hours light/10 hours dark cycle and were allowed to eat and drink *ad libitum*. In experiment 2, quail were divided into 3 groups of ten birds: a control group (C), a low DES treatment group (L) and a high DES treatment group (H).

**Sperm Diluent**

Dulbecco’s Phosphate Buffered Saline (D-PBS) or Lake’s solution (Lake, 1960), widely used as a diluent for chicken semen, were used in our study.

**Collection of cloaca fluid from male quail**

Cloaca fluid was collected from several male quail, and then centrifuged at 8,000x g for 1 hour. The supernatant was used as cloaca fluid.

**Sperm motility analysis**

Sperm motility was analyzed by a CASA system (Hamilton Thorn Research, Beverly, MA, USA). Various system combinations were examined to identify the best condition to examine the motility of chicken and quail sperm.

**DES administration to quail**

Stock solutions of DES (GL Sciences Co., Tokyo, Japan) were prepared by dissolving 1 and 10 mg/ml of DES (L and H dose respectively) in sesame seed oil. The quail were given 100 µl daily injections of the DES solution (groups L and H) or sesame
seed oil (group C) intramuscularly for 7 days. Therefore, 0.1 and 1 mg of DES on a daily basis for a week were administered to the birds of groups L and H, respectively. All quail were examined on the day following the final injection.

**Histology of the testes**

The left testes were taken from all members of each group. They were fixed in 10% buffer (PBS) formalin, dehydrated by passage through a grade series of alcohol and cleared with xylene before embedding in paraffin wax. Paraffin sections (6 μm in thickness) were stained with hematoxylin and eosin (HE staining) according to standard protocols.

**Statistical analysis**

All percentage data on sperm motility were subjected to arcsin transformation prior to statistical analysis. One-way analysis of variance followed by Fisher's protected least significant difference post-hoc test was used to analyze the difference in testicular weight between the three groups. Differences of sperm motility and progressive motility between C and L groups were examined by student's t-test using StatView software (Abacus Concepts Inc, Berkeley, CA). Significance was taken when p < 0.05.

**Results**

**Experiment 1 : Assessment of sperm motility using a CASA system**

Initially, we attempted to collect semen by abdominal massage method (Bogdonoff and Shaffer, 1954), technique widely used to collect chicken semen. However, the number of sperm collected by this method was not sufficient for the CASA system. We choose an alternative method to collect quail semen samples. The quail were sacrificed by decapitation, and the backsides of cloaca were incised. The magnum of the ductus deferens was compressed by fingers (after confirmation of the nipple of the ductus deferens) and semen was squeezed out from the nipple. Using this approach, we were able to collect 1 μl or more semen from each male and this was sufficient to perform the sperm motility assay using the CASA system.

A 1 μl aliquot of semen was diluted 40 times (v/v) with either D-PBS or Lake's solution. Whilst we anticipated Lake's solution would be better than D-PBS we discovered that sperm motility was extremely poor in both solutions. Furthermore, there was a significant amount of clumping. Given this situation, it was necessary to modify the diluent in an attempt to increase motility and reduce clumping.

Caffeine, which is often used to improve sperm motility in other species, was added to Lake's solution at various concentrations (0.5, 0.05, 0.005%, w/v) and then used to evaluate its effect on quail sperm motility. A concentration of 0.005% caffeine was found to be the most effective at improve sperm motility. At higher concentrations, the sperm rapidly lost motility. Based upon these observations, we choose to add 0.005% caffeine to the diluent for our subsequent studies. To circumvent the problem of sperm aggregation, we tried adding cloaca fluid to the diluent. It was found that the addition of 10% (v/v) of cloaca fluid to the diluent significantly reduced sperm aggregation. The best conditions for CASA analysis were therefore established: a 40 x dilution of the semen sample in Lake's solution supplemented with 0.005% caffeine.
Table 1. Effects of DES administration on testicular weights

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Testicular weight (g/100 g BW)</th>
<th>Left</th>
<th>Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.41±0.42</td>
<td>1.29±0.34</td>
<td></td>
</tr>
<tr>
<td>Low group</td>
<td>0.95±0.77*</td>
<td>0.78±0.66*</td>
<td></td>
</tr>
<tr>
<td>High group</td>
<td>0.15±0.11*</td>
<td>0.23±0.33*</td>
<td></td>
</tr>
</tbody>
</table>

1 Control, Low and High groups received sesame oil, or sesame oil containing 0.1 mg or 1 mg DES, respectively.
2 Value of correction for every 100 g in body weight (mean±SEM, n=10).
3 Values significantly different from control group (p<0.05).

and 10% cloaca fluid. Using this as diluent, at least 80% of the sperm were motile. The average concentration of sperm collected from the nipple of the ductus deferens was approximately 7,500 million/ml (n=10).

Experiment 2: Effects of DES administration on male quail's reproductive function

The effects of DES administration on the testicular weights are given in Table 1. The mean values of left and right testicular weights in the C group were 1.41 and 1.29 g/100 g BW, respectively. The weights in the L and H groups significantly decreased in comparison with those of the C group (p<0.05). The differences between the C and H groups were significant (p<0.05).

The results of histological observation on the testes structure are shown in Fig. 1. In control quail, the seminiferous epithelium consisted of several cell layers, and many sperm attached to the seminiferous epithelium were found. On the other hand, seminiferous tubules of the DES treated individuals (both L and H groups) displayed regression. In group L, the lumen of the tubules were narrow and contained only a small number of sperm. The seminiferous tubules of group H displayed a greater degree of regression including disintegration of epithelial cells and complete absence of sperm.

Table 2 shows the effects of DES administration on male reproductive function. The sperm could be collected from all quail in the C group (The recovery rate was 100%). However, the rate of the quail from which sperm could be collected was 50% in the L group, and it further decreased to 10% in the H group. Motility (percentage of motile sperm with a path velocity (12 μm/s)) and progressive motility (percentage of motile sperm with a path velocity (25 μm/s) and moving in a straight line 80% of the time) in group L were significantly lower than those of group C (p<0.05). Only one male from group H yielded sufficient sperm for CASA analysis and these sperm display poor motility and progressive motility.

Discussion

In this report, we show that injection of DES (an estrogenic compound) into
Fig. 1. Effects of diethylstilbestrol administration on the structure of the testes. HE staining. E = seminiferous epithelium, L = seminiferous lumen. Arrows show examples of sperm. Scale bars represent 50 μm. (a) Section of the testis from the control group. The seminiferous epithelium shows well-organized structure with numerous sperm in the seminiferous lumen. (b) Section of the testis from group L. Only a few sperm are observed. (c) Section of the testis from group H. Note the marked disintegration of seminiferous epithelium and absence of sperm.

Matured male quail causes regression of the reproductive organs and a reduction in sperm motility. Further, we define the conditions under which CASA sperm motility analysis performs on quail.

Quail sperm motility could be analyzed by the CASA system. To perform this analysis semen was diluted 40 times in Lake's solution supplemented with 0.005% caffeine and 10% quail cloaca fluid. Identification of a suitable diluent for quail sperm should facilitate additional studies into the factors affecting male fertility.
Table 2. Effects of DES administration on male reproductive function

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No of treated quail</th>
<th>Recovery rate(^1) (%)</th>
<th>Motility(^2) (%)</th>
<th>Progressive motility(^3) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>10</td>
<td>100</td>
<td>83.9± 5.7</td>
<td>33.1± 6.4</td>
</tr>
<tr>
<td>Low group</td>
<td>10</td>
<td>50</td>
<td>34.4±20.6*</td>
<td>16.2±10.6*</td>
</tr>
<tr>
<td>High group</td>
<td>10</td>
<td>10</td>
<td>33</td>
<td>13</td>
</tr>
</tbody>
</table>

\(^1\) Control, Low and High groups received sesame oil, or sesame oil containing 0.1 mg or 1 mg DES, respectively.

\(^2\) Percentage number of quail from which sperm could be collected compared with control group.

\(^3\) Percentage of motile sperm moving with path velocity >12μm/s.

\(^*\) Percentage of motile sperm moving with path velocity >25μm/s and in a straight line over 80% of the time.

\(^{1,3}\) Values of motility and progressive motility are mean±SEM (n=10, 5, and 1 in control, Low and High groups respectively).

* Significantly different from the value of control group with in the same column. Student’s t-test (p<0.05).

Recently, reproductive disorders caused by endocrine disruptors have been reported in a number of animal species (Khan et al., 1998; Atanassova et al., 1999; Stoker et al., 2000). However, little is known about the endpoint of endocrine disruptor action in birds. The present study goes some way towards addressing this issue by examining the effect of DES on adult quail reproduction.

Injection of adult quail with DES caused a significant reduction in testicular weight, number of sperm recovered rate and their motility. In addition, testis pathology revealed a marked disintegration of the seminiferous epithelium and substantial decrease of sperm in the seminiferous lumen. Williams et al. (2001) and McKinnell et al. (2001) demonstrated that suppression of androgen action (reduction of androgen receptor expression and Leydig cell volume in the testis) caused gross abnormalities of the reproductive tract (e.g., reduction of testis weight and reduction in epithelial height of the vas deferens) in male rats treated neonatally with DES. They also concluded that reproductive tract abnormalities induced in the neonatal male rat by a high dose of DES were associated with a grossly altered androgen:estrogen balance (low androgen + high estrogen). Therefore, it was assumed that DES might alter the androgen:estrogen balance of male quail, would have the same effect as it did in mammals.

Yoshimura et al. (2000) examined the effects of DES intake (drinking water containing 0.01 mg/l or 1 mg/l) during their growth phase (from Day 0 of post-hatch to Day 53) on the reproductive organs of Japanese quail. They demonstrated that the weight of the testes and ovaries of DES treated quail at 28-day old were significantly smaller than the controls. However, DES intake did not affect gonadal structures and reproductive function in matured male and female quail. In contrast, the current study showed that DES exposure in adults has a dramatic effect on reproductive
function. The differences in the results between these two studies might be due to the differences in administration of DES and the age of quail.

In conclusion, we suggest that regression in the testis and spermatogenesis (including a decline of sperm motility) occur as the endpoint of endocrine disruptors in matured male quail. These reproductive disorders could lead to a reduction of fertility. In addition, the current study proposed an appropriate method for analysis of sperm motility in quail.

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References


