EGG PRODUCTION AND FERTILITY FOLLOWING VARIOUS METHODS OF INSEMINATION IN JAPANESE QUAIL (COTURNIX COTURNIX JAPONICA)

B. C. WENTWORTH AND W. J. MELLEN

Department of Poultry Science, University of Massachusetts, Amherst, Massachusetts, U.S.A.

(Received 23rd January 1963)

Summary. A modification of the method of Burrows & Quinn (1939) was successfully used to collect semen from male Japanese quail, and females were artificially inseminated by intravaginal, intraperitoneal and intrauterine routes. Others were mated naturally. Semen from antibiotic-fed males, diluted with quail-egg albumin containing antibiotics, and deposited in the uterus (shell gland) by means of a hypodermic needle passed through the egg contained therein, fertilized more than 75% of the females for a mean duration of 4-6 days. This procedure also resulted in excellent egg production and caused no mortality. In contrast, the other methods of artificial insemination resulted in much lower fertility and egg production and, in some cases, heavy mortality.

INTRODUCTION

The Japanese quail (Coturnix coturnix japonica) is gaining widespread favour as a pilot animal among investigators in avian genetics, nutrition, and physiology. In our own case, this species seemed well suited, in many respects, to certain proposed studies involving effects of various treatments on fertility. In particular, we wished to measure duration of fertility following a single insemination, which requires use of artificial insemination in order that one may standardize sperm dosage and know the time of insemination with certainty. However, perusal of the scant literature on artificial insemination in Coturnix was discouraging. Wilcox & Clark (1961) produced hybrids between domestic fowl cocks and female Japanese quail, but were unable to make the reciprocal cross because they could not obtain semen from the male quail. Wilson, Abbott & Abplanalp (1961) state that their modification of the Burrows & Quinn (1939) method for artificial insemination in poultry results in only about 10% fertility when applied to Japanese quail.

A satisfactory method of artificial insemination had to be developed before the Japanese quail could be used in our contemplated research. The present report describes the results of our work on this problem, including a method of semen collection and insemination which does not interfere with egg production and results in at least 75% fertility.
Japanese quail were reared in modified chick-starting batteries and fed quail starter ration ad libitum (modified turkey starter of Consuegra, 1963) until 5 weeks of age. The males and females were then separated and fed commercial turkey breeder ration thereafter. At 7 weeks of age the females were placed in individual compartments in laying cages designed for quail (Georgia Quail Farm, Savannah, Georgia). The lights in the windowless experimental room were controlled by a time clock which allowed 14 hr of light and 10 hr of darkness each day.

When natural matings were used, each female was placed in a cage with a single male for 16 hr from 4 p.m. to 8 a.m.

**Semen collection and dilution**

All feed and water were withheld from the males at least 12 hr prior to semen collection, in order to minimize contamination of the semen with faeces and urine. Collection was timed so that no more than 30 min elapsed between collection and insemination. The use of a bird-holder (Pl. 1, Fig. 1) for collecting semen freed both hands for manipulation. The frothy secretion of the cloacal gland (Coil & Wetherbee, 1959) was forced from the gland by a motion with the left hand, while the secretion deposited at the vent was cleared with the right hand, with a clean towel. The second finger of the right hand was placed below the pubic bones and slight pressure was applied upward. Pressure was applied laterally to the cloacal region with the thumb and index finger of the left hand. The semen thus extruded from the vasa deferentia was drawn up into a 0.25-ml pipette, assembled with a rubber tube and mouthpiece, which was positioned by the thumb and index finger of the right hand while pressure was still being applied by the second finger. Semen from each male was immediately mixed with 0.5 ml of diluent and the diluted samples were then pooled. Spermatozoa in the pooled samples were then counted by the haemocytometer method described by Allen & Champion (1955). This cell count was the basis for determining the final dilution, which contained 40 million cells per 0.1 ml of inseminate, the volume used for insemination in all cases.

Preliminary artificial insemination trials, making use of the Van Drimmelen (1951) intraperitoneal method and the intrauterine method described in detail later, resulted in low fertility, high frequency of soft-shelled (prematurely laid) eggs, high mortality, and frequent cessation of production in survivors. These results suggested that bacterial infection was developing shortly after insemination. Subsequent isolation of *Escherichia coli* from the oviducts, obviously introduced as a semen contaminant, led to the use of antibiotic treatment of males and their semen in further work involving the intrauterine route. For this phase only (intrauterine insemination), the semen collected in the pipette was immediately placed in one of two diluents: (1) Krebs-Ringer diluent (Lardy & Phillips, 1943) with 400 units of penicillin and 500 μg of dihydrostreptomycin added to each ml; (2) quail-egg thin albumin, which was reduced to pH 7.5 with phosphate buffer and then to pH 6.8 with citric acid (Xumsai, 1959).
Fig. 1. Holder used to immobilize the male during semen collection and the female during insemination. (6) Cloacal gland.

Fig. 2. Reproductive organs of the female Japanese quail, in relation to route and site of intrauterine insemination. (1) Hypodermic needle, (2) egg with partially formed shell, (3) utero-isthmus junction, (4) magnum of the oviduct, (5) ovary with developing ova.

(Facing p. 216)
Each millilitre of this diluent also contained 200 units of penicillin and 250 µg of dihydrostreptomycin. In addition, males used in this phase of the study were fed breeder ration containing 500 g of terramycin per ton for a 12-hr period starting 24 hr prior to semen collection.

**ARTIFICIAL INSEMINATION**

Three methods were used: (1) the intravaginal insemination method of Burrows & Quinn (1939), (2) the intraperitoneal technique of Van Drimmelen (1951), and (3) the intrauterine method developed by one of us (B.C.W.) and described below. The Krebs semen diluent mentioned above was used with all three methods, but only with method (3) was antibiotic treatment of male birds and their semen employed. Intrauterine insemination was also done with semen diluted with quail-egg albumin and buffer, plus the antibiotic treatments, as described previously.

Intravaginal inseminations were performed at 6 p.m., following oviposition. Intraperitoneal and intrauterine inseminations were done at approximately 9 a.m., or about 6 to 8 hr before the normal time of oviposition.

Females to be inseminated by the uterine route were placed in the quail-holder (Pl. 1, Fig. 1) and the thumb and index finger of the right hand were placed just anterior to the egg with incomplete shell in utero. A 1½-in., 22-gauge needle, fitted to a 1-ml tuberculin syringe, was inserted about ¼ in. above the vent and through the postero-dorsal end of the uterus and the egg. The needle was passed through the interior of the egg and barely through the antero-ventral end of the shell, where the diluted semen was released (Pl. 1, Fig. 2). Thus the semen was deposited in the anterior portion of the uterus near the junction of uterus and isthmus.

**COLLECTION AND TREATMENT OF DATA**

The percentage production for each hen was calculated as the total number of eggs laid during a test period relative to the total number of days in the period. The eggs were set within 24 hr of laying and then broken out and examined macroscopically for embryonic development after 72 hr of incubation at 102° F in a Buffalo still-air incubator. The duration of fertility following a single insemination was calculated as the number of days from the first day after insemination until the last fertile egg was laid. A test period was terminated only after at least four infertile eggs were laid consecutively. Since all artificial inseminations were made in the morning, except those done by the intravaginal route, the eggs laid 30 to 40 hr later were usually fertile.

Where feasible, statistical analyses to determine the significance of mean differences were carried out (analysis of variance and Hartley's sequential test; Snedecor, 1956).

**RESULTS**

The volume of semen collected from Coturnix males was small, approximately 10 µl per bird. Average sperm concentration was 1·2 million ± 44 thousand spermatozoa per mm³ before dilution, or about 12 million per ejaculate.
As mentioned previously, when the peritoneum or the uterus was the site of semen deposition, and when no antibiotics were used, egg production and fertility were low and many hens died within 1 or 2 days following insemination. Application of standard microbiological techniques in these cases led to isolation and identification of *Escherichia coli* in the oviducts. When the males and their semen were treated with antibiotics, however, *E. coli* was only occasionally detected.

**Table 1**

PRODUCTION, FERTILITY AND MORTALITY OF NATURALLY-MATED AND ARTIFICIALLY-INSEMINATED QUAIL

<table>
<thead>
<tr>
<th>Method of insemination</th>
<th>No. hens tested</th>
<th>Hens fertile* (%)</th>
<th>Mean duration of fertility† (days)</th>
<th>Mean % production of hens laying at least one egg</th>
<th>Mortality* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mated naturally</td>
<td>70</td>
<td>54-3</td>
<td>5-1</td>
<td>72-4†</td>
<td>1-4</td>
</tr>
<tr>
<td>Intravaginal, Krebs diluent</td>
<td>26</td>
<td>23-1</td>
<td>5-8</td>
<td>63-6</td>
<td>7-7</td>
</tr>
<tr>
<td>Intraperitoneal, Krebs diluent</td>
<td>20</td>
<td>45-0</td>
<td>6-2</td>
<td>61-7</td>
<td>40-0</td>
</tr>
<tr>
<td>Intrauterine, Krebs diluent</td>
<td>24</td>
<td>18-8</td>
<td>3-0</td>
<td>34-2§</td>
<td>33-3</td>
</tr>
<tr>
<td>Intrauterine, Krebs diluent, antibiotic treatments</td>
<td>63</td>
<td>50-8</td>
<td>3-3</td>
<td>56-9</td>
<td>3-2</td>
</tr>
<tr>
<td>Intrauterine, albumin diluent, antibiotic treatments</td>
<td>59</td>
<td>77-5</td>
<td>4-6</td>
<td>75-8†</td>
<td>0-0</td>
</tr>
</tbody>
</table>

* No statistical tests done on these data.
† Excluding birds which laid no fertile eggs.
‡ Significantly larger than the other four values in this column; *P*<0.01.
§ Significantly smaller than all other values in this column; *P*<0.01.

Pertinent data comparing the results of various methods of insemination are summarized in Table 1, and need not be repeated here.

**DISCUSSION**

The results presented demonstrate that semen collection and artificial insemination can be accomplished, with good results, in the Japanese quail. Most successful in terms of egg production, fertility, and viability was the intrauterine method, using semen extended with quail-egg albumin and buffer plus antibiotic treatment of both male birds and their semen. The intrauterine method, with antibiotics, was not as successful when the Krebs-Ringer diluent was used. It should be mentioned that our method of intrauterine insemination always caused premature expulsion of the egg which was in the shell gland at the time of insemination.

Since intravaginal insemination gave poor fertility, both in our work and in that of Wilson *et al.* (1961), and because intraperitoneal and intrauterine insemination without antibiotic treatment caused *E. coli* infection, the importance of antibiotics in the eventual development of a successful procedure cannot be over-emphasized. It should be noted here, since not previously mentioned, that when sterile saline solution was used in place of semen, and the intraperitoneal insemination procedure was followed, there was no mortality and no reduction in egg production.
Intrauterine insemination without antibiotic treatment not only caused heavy mortality within 24 hr, but also resulted in a high incidence of soft-shelled eggs and the complete cessation of egg-laying in many of the survivors. It is our opinion that these effects on egg production were due to localized oviduct infection, and not to a general systemic infection, because those birds which did not succumb soon after insemination remained healthy and vigorous, as judged by general appearance and activity. It is likely that such infection blocked ovulation, in many cases, by stimulation of the neurohumoral mechanism demonstrated in the fowl by Huston & Nalbandov (1953), assuming that such a mechanism is not unique to the chicken. This supposition is supported by results of one experiment, unfortunately characterized by excessive mortality, in which three out of four ‘blocked’ quail hens were caused to ovulate, after 7 days of non-production following insemination, by intravenous injection of 0.05 mg of bovine luteinizing hormone (Armour). The injections were made at 9.30 a.m., a time calculated to cause ovulation and oviposition earlier than they normally occur; and the three birds which responded laid normal, hard-shelled eggs before noon of the following day. (Normal laying time is 3 to 6 p.m.)

Since natural mating and intravaginal artificial insemination do not cause difficulties of the kind related in the preceding paragraph, it seems evident that the utero-vaginal junction in Coturnix—and undoubtedly in all birds—is an effective barrier to the passage of bacteria while allowing passage of spermatozoa. Whether the bacterial barrier is chemical or mechanical, or both, is not known; but the work of Allen & Grigg (1957) with chickens, showing that dead spermatozoa deposited in the vagina did not pass into the uterus, suggests that there simply is no mechanism by which relatively non-motile cells can be propelled through the utero-vaginal junction.

The data on sperm density in cock and turkey semen, as compiled from the literature by Nalbandov (1958), yield ‘most commonly observed’ values of 4 million and 7 million per mm$^3$, respectively. The present study shows that the concentration of spermatozoa in Japanese quail semen (about 1.2 million per mm$^3$), at least as collected by our method, is much lower.

Mean duration of fertility in our quail (Table 1) was only 50% or less of that reported for chickens and turkeys, and shorter by a few days than values given for ducks and geese (e.g. Gowe & Howes, 1956; Maw & McCartney, 1956; data reviewed by Smyth & Jeffrey, 1960; Taneja & Gowe, 1961). Since there are no significant differences among the mean duration values shown in Table 1, we can conclude that artificial insemination results in a duration of fertility as great as that following natural matings.

ACKNOWLEDGMENT

The authors gratefully acknowledge the valuable contribution made by Dr Glenn H. Snoeyenbos, Department of Veterinary Science, who isolated and identified the micro-organisms found in the oviducts following intraperitoneal and intrauterine insemination.
Males used in the present study were 7 to 10 weeks of age. In subsequent experiments with older quail (10 weeks to 6 months), semen volume averaged approximately the same as reported here, but mean sperm concentration was much greater—5-9 million/mm³.

REFERENCES