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FOURTH CHAPTER.

A.- SUMMARY.

Literature.

1. Previous work, that has led up to the present knowledge of artificial insemination of birds, is reviewed.

Intraperitoneal Insemination.

2. The technique of intraperitoneal insemination in pigeons and fowls is described. Data collected on-

- (a) 62 intraperitoneal inseminations with 21 control matings of pigeons; and
- (b) the first 117 intraperitoneal inseminations with 33 control inseminations or matings of fowls, which included 3421 "Hen-days" of observation before and after the operation, as well as 978 "Hen-days" of observation before and after the control inseminations or matings, showed the following results:—
 - (1) The egg-production of the female birds was not seriously affected, but in the case of fowls it was temporarily inhibited after the operations, similar to the results of the artificial insemination by other methods (controls). A characteristic notch in the curve of recovery is reported and an explanation suggested.
 - (2) The health of females was not affected by the operations, although under unfavourable conditions a few deaths from peritonitis occurred.
 - (3) In fowls and pigeons the onset of fertility was usually immediate, i.e. the first ovum to be liberated after the operation was as a rule fertile if fertility was produced at all.
 - (4) The length of the period of fertility in the fowl was similar to that reported by other workers after separation of the cocks from the hens, but the average length of the period in the 45 consecutive cases of fertility tested for duration (10.78 days) was slightly less than in the thirteen control cases fertilized after coitus or artificial insemination *per vaginam* (13.85 days). The maximum duration (25 days) however, exceeded by three days the maximum recorded locally in controls, although the maximum reported in the literature, 32-34 days, (Crew, 1926; Nalbandov and Card, 1943; Barfurth, 1896) was not attained.
 - (5) The number of eggs fertilized from one injection in the fowl (4.78), showed an average figure below that of the control cases (6.69), in which other methods of insemination had been employed but the maximum on record (fourteen eggs; Nicolaides, 1934) was exceeded in three out of the 46 cases tested: Sixteen fertile eggs were obtained once and fifteen twice. In the thirteen control cases the number of eleven fertile eggs was the maximum.

- (6) The percentage of fertility of fowl eggs laid during the period between 24 hours after injection of semen and the laying of the last fertile egg in the 46 cases tested, was slightly higher (82.7 per cent.) than in the fertile control cases (82.0 per cent.).
- (7) The decline in percentage of eggs fertile after the middle of the fertile period in fowls was only barely detectable in the cases of intraperitoneal insemination, whereas in the control cases, it amounted to a 50 per cent. drop (Table 26). Fertility during the second half of the fertile period was considerably higher after intraperitoneal insemination than after other methods tested. (64 per cent. against 44 per cent.). The fertile periods of different lengths showed no difference in fertility following intraperitoneal insemination (Table 25).
- (8) The percentage of inseminations proved fertile in fowls was measurably higher after intraperitoneal inseminations than after insemination *per vaginam*.
- (9) The hatchability of fowl eggs after intraperitoneal insemination did not as a rule differ from that found after other methods of obtaining fertility, except that in several individual cases chicks were hatched from eggs laid a few days later after the day of introduction of the fertilizing semen into the hen, viz.: Twentyfourth day twice, twenty-first day once and the twentieth day thrice, than had been recorded in the Eterature (nineteenth day: Nalbandov and Card, 1943).
- (10) The number of chicks hatched from one intraperitoneal insemination reached a maximum of thirteen, with an average of $3 \cdot 3$ in the 36 consecutive cases in which hatchings were recorded. In the 12 control cases the maximum was seven and the average $1 \cdot 7$ chicks.
- (11) In fowls the seasonal influences on the results were strikingly absent, under the given conditions; but the results on hens kept indoors, showed a decreased fertility, which was slightly compensated for by a better hatchability of the fertile eggs collected indoors.
 - (12) Effects of breed, age (or conformation), on previous production and on previous insemination in fowls show that
 - (a) fertility was better in birds of the "heavy" than in birds of the "light" breeds, but this lower fertility in the "light" hens was compensated for to some extent by a larger number of chicks obtained per dozen fertile eggs incubated;
 - (b) second-year hens performed better than pullets in respect of the number of inseminations followed by fertility and hatchings and in percentage fertility, But pullets showed better hatchability and duration of fertility;
 - (c) birds with a production of over 60 per cent. in the ten days prior to intraperitoneal insemination showed all round superior results over birds with a production below 60 per cent.; but the lower fertility of birds with a less than 60 per cent. previous production was again partly compensated for by higher hatchability. The egg-production of the higher producers was more seriously affected.

(13) The influence of the instruments used for the intraperitoneal operation was variable in respect of egg-production and incubation results, viz.: as far as the shape of the penetrating instruments was concerned, those with less adverse influence on production: "blunt" and "fine" needles, were followed by incubation results inferior to the results from the use of "sharp" and "coarse" instruments.

The contact between semen and metal parts was shown to be a factor grossly implicated in the selection of instruments for the intraperitoneal insemination technique. The elimination of metal from the syringes used, was highly favourable to the incubation results, but the wax-coating of the metal needles showed no marked benefit.

The contrast between the positive correlation between fertility and hatchability percentages in connection with factors concerning the semen and the negative correlation between the fertility and hatchability encountered in connection with factors concerning solely the hen is submitted for further investigation.

The conclusion is reached that all-glass (non-metal) instruments with passages of a relatively wide lumen (e.g. 1 mm.), and with blunt penetrating ends, were most suitable for the technique of intraperitoneal insemination. Two special instruments evolved viz.: (a) an all-glass syringe with long glass nozzle (rather fragile) and (b) a modified "Holborn" sheep inseminator made to fit an all-glass insulin syringe, are described.

- (14) The best site for performing the operation in the fowl was a point on the abdominal wall, at the anterior border of the left pubic bone at the ventral border of the superficial muscles that pass over its posterior process. In pigeons the obturator fossa was the site of choice in view of the greater extent of the lateral air-sac.
- (15) The optimum depth of penetration from the best site was $6 \cdot 0$ cm. for smaller hens (e.g., White Leghorn pullets) and $8 \cdot 0$ cm. for larger birds.
- (16) The optimum direction of penetration was in a plane parallel to the backbone in an antero-medial direction at a ratio of 2:1, i.e.: 6.0 cm. forward for every 3.0 cm. in a medial direction.

Semen Collection and Examination.

3. Observations were made on 1,163 collections of pigeon semen of which 805 were made by means of a new technique whereby excitable birds could be more satisfactorily controlled. The method was a modification of that described by Burrows and Quinn (1935) as adapted to pigeons by Owen (1941).

The methods of semen collection from male fowls by fixing a receptacle to a male cloaca during coitus (Parker 1939) and by manual ejaculation (Burrows and Quinn 1935, 1937) were carried out, and a new modification of the latter evolved, by which 292 consecutive collections were executed of which 98.6 per cent. were successful. An average of 0.45 c.c. semen per collection was obtained by this method including the primary failures, and no assistance was required for the holding of the birds or the semen receptacles. A wire leg-holder for the control of birds without assistance was evolved.

(a) The semen of pigeons had a density of approximately 2 million sperms per c.mm. when collected with a pipette direct from the vent. The volume obtained averaged 0.005 c.c. A relatively small number of racing homer pigeons proved to be good semen producers. There was a very large variation between individual birds and separate collections. One bird produced markedly pathological spermatozoa for a time.

(b) The semen of fowls was measured, examined and tested during all seasons in the *first, third* and *fourth* series of inseminations, using a variety of males which supplied semen with—

- (1) an average volume of 0.45 c.c. per collection;
- (2) a viscosity which varied in different males and in the same male at different collections, from a watery semen to a thick oily semen, estimated to run 0.25 times as fast as water through a glass tube of 0.2 mm. bore at room temperature;
- (3) the colour of pure semen was as a rule ivory white;
- (4) the percentage of samples soiled varied in different males to average about one quarter, when untrained males were included;
- (5) motility in fresh samples at room temperature was almost 100 per cent. progressive in all samples tested.
- (6) The estimated density of the samples varied between one million and 5.5 million sperms per c.mm. but in the small number of samples checked by haemocytometer counts, the density was found to be usually nigher than the rough estimate from semen smears, 7.9 million per c.mm. being the maximum counted. Two samples counted by the technique of milk-" Breed "-clump-counts, showed a figure of 8.5 millions.
- (7) the pH measurements on semen by the "B.D.H. Capillator" gave readings of 7.1 to 7.4. Accurate determinations with "Beckman's pH meter" gave readings of 7.0 to 7.4;
- (8) morphological abnormalities were very rarely observed and only occurred in large numbers in the semen samples from a very young cockerel and a relatively infertile but not sterile cock. The most striking abnormality noticed was the curled up head which was often also seen in semen samples aged *in vitro*.
- (9) the fertilizing qualities of semen samples were tested in a total of 220 intraperitoneal inseminations and 71 control matings and inseminations on 81 hens used in the *first, third* and *fourth* series of inseminations with the following results:--
 - (i) The fertility of semen samples inseminated by the intraperitoneal method was higher for most males, than from samples introduced into the vagina, and the duration and percentage hatchability in the largest group (cock No. 6 Table 48), was also higher for intraperitoneal insemination.
 - (*ii*) Larger doses of semen were followed by better fertility and a fertile period of longer duration. (Table 49.)

- (*iii*) The samples of semen subjected to storage and dilution gave a markedly lower fertility than fresh samples (Tables 51 and 52) although storage up to 2 hours under liquid paraffin had little effect.
- (*iv*) The results of insemination with mixed somples of semen were often inferior to those with pure separate semen and provided some evidence that a mixed semen sample tends to be reduced to the quality of the poorest of its components, rather than that the poor quality of a given sample of semen can be offset by admixture with semen of better quality. Except in two isolated instances in the third series of inseminations all the chicks hatched from each insemination with a mixed sample of semen, were the progeny of only one of the males which contributed semen to the sample.
- (v) Simultaneous insemination with different samples of semen, with different instruments by the intraperitoneal method had results in agreement with the findings mentioned earlier:
 - (i) Deposition of the semen in the region of the ovary was more successful in producing fertility, than deposition in the posterior peritoneal cavity near the point of entry, irrespective of which was the larger dose.
 - (ii) The coarse needle was more favourable to successful insemination than the fine needle (Table 56).
 - (iii) Pure semen produced fertility although injected through a fine needle, whereas mixed semen injected through a coarse needle into the same bird at the same time, failed (Table 57) to be successful.
- (vi) Insemination with different samples of semen by the various methods showed that—
 - (i) the fertilizing quality of semen was more important than the route of introduction in determining which of two kinds of sperm would gain the advantage in the competition for fertilization of ova when present in the hen at the same time (table 58);
 - (ii) the same was true in respect of the doses of semen used, unless smaller doses than 0.1 c.c. were given;
 - (iii) the superior fertilizing quality of a given semen sample, was lost on admixture with a sample of indifferent quality.

Storage of Spermatozoa in the Hen.

4. Storage of fowl spermatozoa *in vivo* in the body of the fertile hen was studied by examinations made on thirty-three hens during the *first*, *third* and *fourth* series of inseminations. A new technique was evolved whereby sperm-cells could be picked up from serous and mucous surfaces by means of capillary action of small, very fine, glass tubes.

(1) The lumen of the infundibulum was the only locality where spermatozoa were demonstrated in a morphologically normal and progressively motile state, during the period 3 to 14 days (72 to 336 hours) following insemination by various methods.

(2) The discovery of sperm concentrations contained in the mucosa of the chalaziferous region in the infundibulum in one hen eight days after intraperitoneal insemination has been confirmed by demonstration of similar structures containing sperm in two hens four and six days after natural copulation and separation from the male.

(3) The term "Spermnests" has been proposed for this structure and the significance of this finding is discussed.

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