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EFFECTS OF SPERM CELL NUMBERS, ROUTE OF INSEMINATION, AND DILUTION OF SEMEN ON FERTILITY IN THE DOMESTIC FOWL

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Ronald James DeMeritt

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H C Tindell JHC

Major professor

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EFFECTS OF SPERM CELL NUMBERS, ROUTE OF INSEMINATION, AND DILUTION OF SEMEN ON FERTILITY IN THE DOMESTIC FOWL

By

Ronald James DeMeritt

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ABSTRACT

EFFECTS OF SPERM CELL NUMBERS, ROUTE OF INSEMINATION AND DILUTION OF SEMEN ON FERTILITY IN THE DOMESTIC FOWL

By

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By using intravaginal artificial insemination (I.V.A.I.), intramagnal artificial insemination (I.M.A.I.) and genetic markers, three separate experiments were conducted on the domestic fowl to examine the following: (1) the effect of sperm cell numbers and route of insemination on fertility, hatchability and duration of fertility; (2) the effect of semen dilution on fertility, hatchability and duration of fertility; and (3) movement of spermatozoa into and out of the host glands located at the uterovaginal junction (U.V.J.).

In Experiment I various volumes of viable spermatozoa equivalent to (1) 12.5 million, (2) 25 million, (3) 50 million and (4) 100 million from Single Comb White Leghorn (S.C.W.L.) or Barred Plymouth Rock (B.P.R.) males were inseminated I.V.A.I. or I.M.A.I. into S.C.W.L. hens. The results indicated that S.C.W.L. spermatozoa was superior to B.P.R. spermatozoa's fertilizing capacity at all four spermatozoa number levels of insemination. Fertility measured during the first week of Experiment I indicated that hens inseminated I.M.A.I. with 12.5 million and 25 million viable spermatozoa produced eggs with higher fertility than I.V.A.I.

inseminated hens. Inseminations with 50 million and 100 million viable spermatozoa I.V.A.I. resulted in superior fertility during week one when compared with 50 million and 100 million spermatozoa inseminated I.M.A.I. During weeks two and three following a single insemination, fertility achieved with I.M.A.I. was higher than with I.V.A.I. at all four levels of insemination.

Duration of fertility with I.M.A.I. was longer than with I.V.A.I. to a highly significant degree. The S.C.W.L. spermatozoa provided a significantly longer duration of fertility than the B.P.R. spermatozoa. A linear trend indicated a longer duration of fertility with increased numbers of viable spermatozoa occurred with I.V.A.I. for both breeds and I.M.A.I. for the B.P.R. only. Intramagnal artificial insemination with the S.C.W.L. spermatozoa did not have a linear trend because the 12.5 million and 25 million level of insemination had long durations of fertility similar to the higher levels of insemination.

In Experiment II, in which only S.C.W.L. were used, the results suggested higher fertility was gained during the first week after I.V.A.I. as the neat (pure semen) was diluted with Beltsville Extender to a 1:4 (semen:diluent) ratio. The opposite appeared to be true with I.M.A.I. as indicated by decreased fertility during the first week as the semen was diluted up to 1:4.

During the second and third weeks of Experiment II, hatchability was significantly higher at the 1:1 and 1:4 dilution ratios than with neat semen. Duration of fertility was longest when diluted semen, particularly 1:1 dilution, was inseminated.

In Experiment III, New Hampshire (N.H.) females were inseminated. S.C.W.L., B.P.R. or N.H. sires were identified by progeny phenotypes.

Therefore, the sequence of inseminations with 25 million or 75 million viable spermatozoa I.V.A.I. could be compared to the pattern of sperm cell release from the U.V.J. host glands. All possible two breed combinations were made with inseminations at 0-1 hour or 0-24 hour intervals while all possible three breed combinations were made at 0-24-48 hour intervals.

In Experiment III a definite breed effect on percent fertility, duration of fertility and hatchability was shown with the S.C.W.L. being superior followed by B.P.R. and N.H., respectively. The fertility and duration of fertility were superior with 75 million viable spermatozoa compared to 25 million viable spermatozoa for both the two breed and three breed combinations. Also, in both the two breed and three breed combinations, semen from the last insemination regardless of breed, facilitated higher fertility and a longer duration of fertility than semen from the previous insemination(s). These data suggested the sequence of spermatozoa release from the host glands was opposite to the sequence of introduction. Therefore, the U.V.J. host glands can regulate or contribute to the release of viable spermatozoa as indicated by the lack of complete randomization of the resulting progeny.

To my wife Nancy

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I wish to express my sincere appreciation to Dr. H. C. Zindel for his support and many words of wisdom during my stay at Michigan State University; to Dr. T. H. Coleman for his guidance in the preparation of this dissertation; and to Dr. B. J. Marquez for his research assistance. A special thank you to Terry Wing for his aid in the statistical analysis and to my wife for her encouragement and enthusiastic support.

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INTRODUCTION

The use of artificial insemination (A.I.) has been shown to elicit excellent fertility, hatchability and duration of fertility in the domestic fowl. Uterovaginal host gland tubules and infundibular crypts facilitate sperm storage for intravaginal artificially inseminated (I.V.A.I.) semen and intramagnal artificially inseminated (I.M.A.I.) semen, respectively.

By use of Beltsville Extender in dilutions of 1:1 to 1:4 semen to diluent ratios with 100 million spermatozoa, optimum fertility and duration of fertility are achieved with I.V.A.I. (Sexton, 1977). This is a direct reflection of the storage capabilities of the uterovaginal tubules when fresh semen is used. However, the dilution and number of spermatozoa necessary for peak fertility and duration of fertility using I.M.A.I. have not been determined.

With the advent of new frozen-thawed semen storage techniques, more information is needed about the storage sites and their relationships to sperm numbers and dilution.

The purpose of this research was three fold. The research was divided into three separate experiments accordingly. Experiment I was designed to gain insight as to the capability of the infundibular crypts to handle various numbers and/or volumes of Single Comb White Leghorn or Barred Plymouth Rock spermatozoa during intramagnal inseminations, when compared to the I.V.A.I., which served as a control.

Experiment II was designed to validate Experiment I data and add the effect of different dilutions of viable spermatozoa from S.C.W.L. males on the storage capabilities of the infundibular crypts as measured by fertility and duration of fertility.

Experiment III was designed to examine sperm cell introduction, storage, release and transport in the oviduct with special emphasis on the role of the U.V.J. host glands.

The area of research involving the relationships between sperm cell numbers, dilution of semen, route of insemination, fertility, hatchability, duration of fertility and the role of the U.V.J. host glands and infundibular crypts is just beginning to be understood.

With the advent of new freeze-thaw techniques, new avian wildlife breeding programs, extinction of rare birds, etc., groundwork concerning the previously mentioned relationships needs to be done.

The purpose of this dissertation is to examine these relationships and relate them in such a way as to be advantageous to the poultry industry and other concerns.

REVIEW OF LITERATURE

In the domestic fowl (Gallus domesticus), the passage of spermatozoa up the female reproductive tract to the site of fertilization has received special attention by researchers in past years. Although great strides have been made, more questions about sperm transport have been left unanswered than have been resolved.

The early works in avian reproduction emphasized the importance of proper environmental conditions to obtain high levels of fertility and hatchability. Floor type matings were utilized with different male to female ratios. Brantos et al. (1972) found ratios of 15 females/male for light breeds and 12 females/male for heavy breeds to be satisfactory. This floor mating system was an area of the poultry industry which had drawbacks in disease control, feed efficiency, space utilization and low fertility. Hayes and Sanborn (1939) working with Rhode Island Reds indicated that the infertile matings were caused by the males, because a change in males resulted in 93% of the infertile matings becoming fertile. This emphasized the need for male selection based on semen quality and possibly the need for another type of mating system.

Artificial insemination (A.I.), along with cage rearing of birds, has been shown to overcome some of these problems. While working with turkeys, Stotts and Darrow (1955) achieved increased fertility and thereby increased economical gain when artificially inseminated cage birds were compared with floor matings. Comparing 31,000 hens from 21 flocks,

they found an increase of 7% in high fertility flocks (from 80-85% fertility to 87-92% fertility) and an increase of 12% in low fertility flocks (from 65-70% fertility to 77-82% fertility).

Practical application and increased fertility utilizing artificial insemination in the chicken was demonstrated by Burrows and Quinn (1939), Warren and Gish (1943), Rainford (1956) and Perek (1966). Although the actual outcome of A.I. was higher fertility and hatchability, the physiological events which lead to these outcomes are just beginning to be understood.

Chicken semen was collected as early as 1914 when Payne collected spermatozoa from the cloaca of a hen following mating. Hutt (1929) collected directly from the male with a watch glass during a mating attempt. One of the more practical and expedient techniques was developed by Burrows and Quinn (1937). By a series of stimulations in the latero-abdominal regions, and pressure above the vent, seminal fluid was forced from the posterior vas deferens into a collection tube.

Van Wambake (1976) studied two different methods of semen collection to investigate the influence of a slight contamination of semen with transport fluid resulting from a simplified semen collection technique upon fertility and hatchability. He concluded that transport fluid was not detrimental to fertility.

Fecal contamination of semen by careless collection also decreases semen quality. Boone and Hughes (1970) illustrated the relationships between increased contamination of the sample and decreased sperm motility and vigor, thereby decreasing fertility.

The time and frequency of collection of semen from the males appears to affect its quality. Parker (1949) suggests a collection every five

days to maintain adequate numbers of spermatozoa.

The actual time of artificial insemination or natural mating also has been shown to affect fertility. Moore and Byerly (1942) utilizing artificial insemination noted lower fertility when hens with a hard-shelled egg in the uterus were inseminated compared to the higher fertility of hens inseminated with no egg in the oviduct. Gracewski and Scott (1943) found higher fertility when males were permitted to mate only during the afternoon. Parker (1945) found similar results and showed its relationship to time of lay, indicating inseminations made prior to laying resulted in lower fertility. Johnston and Parker (1970), adding to this information, concluded fertility was lowest when inseminations were made one hour after or four hours before oviposition. They indicated that the best time for A.I. was in the late afternoon or after the majority of eggs were laid. Christensen and Johnston (1975) found similar results working with turkeys. When studying the effect of time of day in which inseminations were made on fertility, they concluded fertility was highest when inseminations were made at 6:00 P.M. and lowest when inseminations were made at 1:00 P.M. or during a period ten hours prior to oviposition.

The volume, dilution and type of extender to be inseminated became an important factor for economic reasons. Munro (1938) studied the effect of the dilution and density of semen on the fertilizing capacity of fowl semen suspensions. He found fertility decreased as the dilution ratio was increased from 1:2 to 1:128 with seminal plasma and Milanov's solution. Lake (1960) performed studies on the dilution and storage of fowl semen indicating the importance of fructose in the extender. Clark (1969) noted increased fertility and hatchability with a diluted semen sample compared to neat semen in turkeys. Clark combined fertility data from

two strains of turkeys and showed that the use of diluent to store semen was not very successful. However, the use of diluent at 0 hours enhanced not only egg fertility but also the hatchability. The overall production of first quality poults, associated with the use of diluent, was enhanced by 13.2 percent.

Allen and Bobr (1955) used glycerol as a diluent and measured fertility when inseminations were made intrauterine and intravaginally. They found intrauterine inseminations made with semen containing 15% glycerol resulted in higher fertility (73%) compared to the intravaginal inseminations with semen containing the same diluent resulting in 0% fertility.

Wilcox and Clark (1962) studied the use of a diluent during storage of chicken spermatozoa. An optimum dilution ratio of 1:2 and 1:4 resulted in higher fertility than dilution ratios of 1:10 or greater which resulted in zero fertility.

The storage of spermatozoa outside the fowl (in vitro) has generated interest. The use of freezing techniques to preserve sperm until a further date has been studied by Allen (1958), who reviewed the work by Polge which indicated the following: (1) the early successes in the field of deep freezing were carried out on fowl semen and were reported by Polge (1951); (2) the incorporation of glycerol in the diluent permitted complete resumption of motility on thawing the sperm from frozen condition, but rendered the sperm infertile even if they had been frozen; (3) the osmotic damage to the sperm in the oviduct was one major cause of the loss of fertilizing power of glycerolized semen. Allen (1958) maintained factors as yet unknown (other than osmotic shock) were involved in the low fertility.

Graham and Pace (1967 a, b) and Harris and Sweeney (1971) studied

biochemical changes and protein concentration in spermatozoa due to freezing while noting their relationships to decreased fertilizing capabilities of the frozen-thawed sperm cells.

Lake (1967) made a reappraisal of A.I. which demonstrated the need for further research in the area of frozen-thawed semen because of the problem of maintaining sufficient spermatozoa in a condition enabling them to survive in the oviduct for long periods after being stored at sub-zero temperatures.

Harris (1968) studied fertility of chickens inseminated intraperitoneally with semen preserved in liquid nitrogen. He noted a decrease in fertility from preserved semen when compared to similar inseminations with fresh semen.

Harris et al. (1973) noted changes in the ultrastructure of fowl spermatozoa due to rapid freeze-thaw. He noted several changes in morphology attributed to rapid freeze-thaw as follows: (1) extensive divergence of the cytoplasmic membrane throughout the entire length of the spermatozoa; (2) complete destruction of the cytoplasmic membrane surrounding the acrosomal cap and (3) release of mitochondria from the midpiece.

Lake (1968, 1972) stored fowl spermatozoa in liquid nitrogen and found that not only are individual hens different in their ability to sustain and permit thawed spermatozoa to fertilize eggs within their oviduct, but that males differ in the ability of their semen to withstand freezing. Lake (1972) also noted glycerol at 2% or lower to be the best cryoprotective agent for the spermatozoa to survive thawing and achieve any fertility after insemination.

The work done with frozen avian semen has expanded greatly over

recent years. Marquez and Ogasawara (1973) found greater fertility using intramagnal artificial insemination compared to I.V.A.I. of frozen-thawed turkey semen.

Freezing semen has great potential in the poultry industry. Further work is required not only in the types of extender, holding temperature, freezing and thawing, but on the actual site of the insemination using frozen-thawed semen in both the turkey and chicken.

In order to fulfill the goal of successful insemination, more work needs to be done on the optimum numbers of spermatozoa to be inseminated I.M.A.I. or I.V.A.I.; the proper dilutions to be used (1:1, 1:4, etc); and the introduction, transport and release of spermatozoa into and out of the storage sites within the oviduct.

Two spermatozoa storage sites have been identified in the domestic fowl hen. The first is the infundibular crypts described by Van Drimmelen (1946). The second storage site is the uterovaginal host glands discussed by Bobr et al. (1962, 1964 a, b). The importance of the tubules located in the host glands was noted when spermatozoa were uniformly found in the tubules of the uterovaginal junction but rarely in the infundibulum after natural mating. These observations suggest that the uterovaginal junction is the primary site of storage of spermatozoa and that a few spermatozoa ascend to the site of fertilization immediately prior to ovulation.

Mero and Ogasawara (1970) studied the anatomy of the host glands and described the sperm storage tubules as being enlarged or nonenlarged with similar lengths (approximately 500 microns) and similar neck widths (approximately 38 microns). They suggested that tubular enlargement was associated with sperm release.

Gilbert et al. (1968) studied the innervation and vascular supply of

the uterovaginal sperm host glands. This work indicated that nerves were not associated with these glands, although nerves were demonstrated in other regions of the oviduct. The glands have a complex blood supply.

This information stimulated questions concerning storage potentials of each area of storage. That is, which site of storage, the infundibulum or the U.V. junction, plays the major role in storage and release of viable spermatozoa?

A new method of insemination (I.M.A.I.) was necessary to study the storage potentials of the U.V. junction and infundibulum. Intraperitoneal insemination in the chicken was done by Van Drimmelen (1951), and Gilbert et al. (1965). This technique, either intraperitoneal or intramagnal artificial insemination (I.M.A.I.), allowed for the introduction of spermatozoa into the infundibular storage area without first passing by or through the uterovaginal junction.

One problem researchers found to exist with I.M.A.I. was the delay of oviposition. Rothchild and Fraps (1945) found that the incidence of follicular atresia and the time before resumption of ovulation following operation were inversely proportional to the rate of ovulation preceeding the operation. Bobr et al. (1965) noted inhibition of ovulation in the domestic hen when intrauterine inseminations were performed.

Olsen (1952), using a series of intraovarian inseminations, provided evidence that fertilization of mature chicken ova occurred in the oviduct and not the ovary.

Allen and Bobr (1955) studied the role of the uterovaginal junction in limiting the numbers of spermatozoa that ascend the oviduct. Ogasawara et al. (1962) expanded upon this idea by inseminating semen from low fecundity cocks, characterized by poor motility, abnormal spermatozoa and

poor fertilizing capabilities when inseminated by standard techniques, and obtained moderate fertility when semen was deposited in the uterus.

Fujii and Tamura (1963) studied the location of spermatozoa in the oviduct of the domestic fowl with special reference to storage of sperm in the vaginal gland. They indicated the majority of sperm are found in the uterovaginal host gland after insemination.

Burke et al. (1969) found sperm transport from the U.V. junction to the site of fertilization occurred even with inhibition of ovulation and oviposition by hormones, although the rate of movement was reduced.

Burke et al. (1972) noted no evidence of a cyclic secretion of material into the lumen of tubules corresponding with the movement of ova in the oviduct and with the time of oviposition.

Harris (1968) studied the effects of cooling rate, equilibration time, and addition of turkey egg yolk to a glutamate diluent, on the fertility of frozen chicken spermatozoa. He stated the highest fertility (77%) was obtained with intraperitoneally inseminated semen that was equilibrated 0.5 hours in a turkey egg yolk diluent and cooled at a rate of 6°C. per minute. The intraperitoneal insemination resulted in a higher fertility than the intravaginal insemination.

Ogasawara et al. (1969) stored semen at 5°C. for various lengths of time and noted fertility after intravaginal (I.V.A.I.) and intramagnal artificial insemination (I.M.A.I.). It was found that I.M.A.I. hens had equally high fertility but for a longer duration, indicating that the uterovaginal junction is an effective barrier to handicapped spermatozoa, which yet are capable of fertilizing eggs if deposited close to the site of fertilization.

The study of fertility in the domestic fowl had been specialized to

the degree that the actual numbers of spermatozoa inseminated affected the fertility, hatchability and duration of fertility. Specialized techniques for estimating the numbers of spermatozoa collected had to be developed. Shaffner and Andrews (1943) used the packed cell volume to estimate semen density. Techniques have been described by Salisbury et al. (1943) for bull semen and used for studying fowl and turkey semen by Jones and Lamoreux (1942), Gowe (1949), Howes (1951), Carson et al. (1955), Kosin and Wheeler (1956), van Tienhovan et al. (1958) and McCartney and Brown (1959). These authors used pooled semen samples from several cocks at known dilutions to determine the relationship between the optical density of semen and the direct count of spermatozoa made by the hemacytometer.

Allen and Champion (1955) noted the relationship between abnormal spermatozoa and competitive fertilization utilizing different breeds of chickens. They studied competitive fertilization using distinct genetic markers. In their study, they found that the number of viable spermatozoa inseminated was different from the actual number of spermatozoa inseminated. Earlier work involving genetic markers in the poultry industry is voluminous. Goodale (1909) (1910), Pearl and Surface (1910), Dryden (1916), Dunn (1922), Hutt (1949) and Jerome and Cavers (1952) laid the groundwork when they studied the genes for black pigment and barring.

Kosin and Wakely (1950) and Harper and Parker (1952), with the aid of genetic markers studied the overlapping of progeny from changing mates in turkey matings. Warren and Kilpatrick (1929), and Warren and Gish (1943) did similar work with chickens. Crew (1926) and Dunn (1927) indicated that artificial insemination twice within a three day interval with semen from replacement males would decrease the male changeover time.

METHOD AND MATERIALS

Experiment I

One hundred and sixty Single Comb White Leghorn (S.C.W.L.) laying hens, fifteen mature S.C.W.L. males, and fifteen mature Barred Plymouth Rock (B.P.R.) males were housed with thirteen hours of continuous light, fed layer-breeder ration (Appendix A), and given clean water ad libitum. All hens in individual cages were shown to be infertile prior to insemination. Semen samples were collected weekly from each male, and tested for quality by the method developed by Ernst and Ogasawara (1970).

Trial IA - The one hundred and sixty hens were divided into twenty groups containing eight birds per group. Twelve groups were inseminated intravaginally (I.V.A.I.) and eight groups were inseminated intramagnally (I.M.A.I.) using pooled semen from S.C.W.L. males or B.P.R. males. Inseminations encompassing different volumes were used equivalent to the following number of viable sperm cells: (1) 12.5 million diluted 1:1; (2) 25 million diluted 1:1; (3) 50 million diluted 1:1; (4) 50 million neat; (5) 100 million diluted 1:1; (6) 100 million neat (Table 1). Beltsville Extender was used as the diluent.

Trial IB - In Trial IB (Table 2) the experimental design was the same as in Trial IA with the addition of two more groups intramagnally inseminated with 100 million spermatozoa from S.C.W.L. or B.P.R. males.

Trial IC - Trial IC (Table 3) was the same as Trial IB with the elimination of 50 million spermatozoa (neat) inseminated intravaginally.

Pre-trial sperm cell numbers were determined from pooled semen by packed cell volume and optical density. The optical density was measured with the Fisher Electrophotometer set with the red filter, 350 millimicrons, see Appendix B for technique.

Once the optical density was known, the number of spermatozoa was counted by use of the hemacytometer. By plotting the actual counted number against the optical density, a linear regression was made for the semen from each breed. The S.C.W.L. and B.P.R. regressions were identical (Appendix C).

Because time was a critical factor prior to the insemination of fresh semen, the actual number of viable spermatozoa to be inseminated was estimated by subtracting an estimated ten percent for dead and abnormal spermatozoa from the number determined by optical density (Appendices B and C). This number of spermatozoa inseminated I.V.A.I. or I.M.A.I. was carefully calculated and volumes equivalent to the above numbers of viable sperm cells were measured and inseminated by use of the Hamilton Microliter Syringe. A live-dead stain was made at the time of insemination.

The same samples utilized for optical density were later taken to the laboratory where counts were made with the hemacytometer. This number minus the actual percent abnormals and dead spermatozoa as determined from the live-dead stain, Ernst and Ogasawara (1970), gave the real number of viable sperm cells in the pooled sample. Differences in the estimated number and real number were insignificant during analysis of the data.

Special care was taken during the insemination to utilize only fresh semen. If longer than ninety minutes were required for testing and insemination, fresh samples were again collected and tested as shown earlier.

The I.V.A.I. was performed by introducing into the area of the U.V. junction, via plastic straw, different volumes of spermatozoa equal to the desired number of viable spermatozoa. This volume of spermatozoa was measured with the Hamilton Microliter Syringe.

The I.M.A.I. was a bit more complex requiring: (1) anesthetic-Brevitol (trade name - Eli Lilly) or Sodium Pentabarbitol; (2) surgical opening of the left lateral wall exposing the magnum; (3) insemination by injecting spermatozoa with the Hamilton Microliter Syringe attached to an 18 gauge needle; and (4) suturing of the incision after insemination. This second route of insemination required the hen to have a hard-shelled egg in the uterus which allowed for easy exposure to the magnum, thus creating less stress on the bird. Both insemination methods were performed simultaneously.

After inseminations were made, the eggs were collected and marked daily with the hen number and date of lay until infertility for five consecutive days was established by examination of candled eggs. After the freshly laid eggs were collected and marked, they were stored between 50°-60°F. until setting which was done weekly. After seven days of incubation at 99.5°F., all eggs were candled for fertility. Those categorized as infertile were broken open for further examination of fertility as described by Kosin (1945). The remainder of the fertile eggs were incubated until the 18th day when they were transferred into the hatcher at 98°-99°F. and 93°F wet bulb reading until hatching. After twenty-one days incubation, all eggs set were categorized as the following: (1) hatched; (2) pipped live;. (3) pipped dead; (4) live in shell; (5) dead in shell; (6) early dead, as seen in Appendix D.

Data from Experiment I Trials IA, IB, IC were analyzed by a modified

one way analysis of variance[1] for fertility weeks 1, 2 and 3; hatchability weeks 1, 2 and 3; and duration of fertility. See Tables 1, 2, 3, 4, 5 and 6 for experimental format and analysis of variance tables, respectively.

Because treatments were different in Trials IA, IB and IC, a separate analysis of variance on each set of data was performed after the arc-sin transformations were made.

As a result of the shorter duration of fertility from I.V.A.I., only data for I.M.A.I. were analyzed for the third week of fertility and hatchability.

Also it was assumed that any zero values for fertility, hatchability and duration of fertility were due to characteristics of the particular hen and not the technique used.

Experiment II

In this experiment one hundred and twenty S.C.W.L. hens in production and fifteen mature S.C.W.L. males were housed in conditions similar to those in Experiment I.

The hens were divided into twenty-four groups that contained five birds per group. Twelve groups were inseminated via the I.V.A.I. route, and twelve groups were inseminated via the I.M.A.I. route using pooled semen from S.C.W.L. males. Twelve treatments encompassing different volumes were used equivalent to the following number of viable sperm cells: (1) 12.5 million neat; (2) 12.5 million diluted 1:1; (3) 12.5 million diluted 1:4; (4) 25 million neat; (5) 25 million diluted 1:1;

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[1]A modified one way analysis of variance was used for analyzing Experiments I, II and III. Further information on analysis can be obtained from Dr. D. L. Cox and Terry Wing, Iowa State University.

(6) 25 million diluted 1:4; (7) 50 million neat; (8) 50 million diluted 1:1; (9) 50 million diluted 1:4; (10) 100 million neat; (11) 100 million diluted 1:1; (12) 100 million diluted 1:4. Beltsville Extender was used as the diluent.

Sperm cell numbers were determined similar to Experiment I, with the optical density (Appendices B and C) being the sole indicator of sperm numbers.

The eggs were collected, marked, incubated and hatched as done in Experiment I.

Data from Experiment II, Trial IIA and IIB were analyzed with a modified one way analysis of variance[2] for fertility, hatchability and duration of fertility similar to Experiment I. See Tables 33, 34A and 34B for format and analysis of variance outlined, respectively. As in Experiment I, a separate analysis of variance was performed on each trial.

Experiment III

Ninety-six New Hampshire (N.H.) hens in production, fifteen S.C.W.L. mature males, fifteen B.P.R. mature males, and fifteen N.H. mature males were housed in conditions similar to those in Experiment I.

The hens were divided into twenty-four groups that contained four birds per group. Twelve groups were intravaginally inseminated with pooled semen equivalent to twenty-five million viable spermatozoa or seventy-five million viable spermatozoa diluted 1:1 with Beltsville Extender at time zero and one hour later. The other twelve groups were intravaginally inseminated with pooled semen equivalent to twenty-five million viable spermatozoa or seventy-five million viable spermatozoa at time zero and

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[2]Refer to Footnote 1 on Page 15.

twenty-four hours later.

All possible breed combinations were utilized to assure that pooled semen used at time zero was not from the same breed used for the second insemination performed one hour or twenty-four hours later (Table 53). This experiment was repeated twice in Trials IIIA and IIIB, respectively.

Viable sperm cell numbers were determined by the same method used in Experiment I with the optical density (Appendix B) being the sole indicator of sperm numbers.

The eggs were collected, marked, incubated and hatched as in Experiment I. However, the experiment required special attention to assure proper identification of pedigree.

A pre-trial test was done to establish the accuracy of the following color characteristics of the newly hatched chicks, from the three possible matings: (1) N.H. male x N.H. female - rapid feathering, red color; (2) S.C.W.L. male x N.H. female - rapid feathering, white and yellow color; (3) B.P.R. male x N.H. female - slow feathering, black color.

All of the offspring showed the respective genetic markings as described above. This pre-trial test was also used to check fertility, hatchability and duration of fertility of the three different crosses.

Trials IIIA and IIIB were analyzed by a modified one way analysis of variance[3] for characteristics which would lead to information concerning the introduction and release of spermatozoa from the uterovaginal host glands. See Tables 53, 54A and 54B for experimental design and analysis of variance. A list of parameters analyzed is in Appendix F.

After the completion of the two breed cross, the experimental design

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[3]See Footnote 1 on Page 15.

was changed to facilitate a three breed cross (Table 55). This would give more insight into sperm introduction and release from the uterovaginal host glands. This experiment was also repeated twice in Trials IIIC and IIID, respectively.

The experimental design (Table 55) again allowed for all possible breed combinations but eliminated the time interval as a variable. Three intravaginal inseminations were made consecutively twenty-four hours apart utilizing either twenty-five million or seventy-five million viable spermatozoa.

See Appendix G for a list of parameters analyzed.

RESULTS

Experiment I

Trial IA

Fertility: Trial IA

During the first week a comparison between 1:1 dilution and neat semen showed no dilution effect on fertility. There was no difference in fertility between various treatments using neat semen (Table 4).

Inseminations of diluted semen resulted in a significant breed effect indicating the S.C.W.L. as having a higher mean fertility of 67.58% compared to the lower B.P.R. mean fertility of 54.66% (Table 7).

The number of spermatozoa inseminated showed an interaction with the route of insemination. As indicated in Table 8, the mean fertility for 12.5 million, 25 million and 50 million viable spermatozoa inseminated was consistent while the 100 million was highest with the I.V. route of insemination.

During the second week no difference in fertility was shown between neat and diluted semen, although within the diluted inseminations, the superiority of the I.M.A.I. route was highly significant. Also the breed and route interactions were highly significant. Table 9 shows the higher fertility of the S.C.W.L. for the I.M.A.I. as compared to the lower fertility with the I.V.A.I. The interaction occurred because the B.P.R. breed did not elicit an increase in fertility with the I.M.A.I.

The number and route interaction during the second week of fertility

(Table 10) showed a linear trend with the I.V.A.I. with 100 million being highest. The I.M.A.I. lacked this linear trend because of the lower fertility utilizing the 100 million spermatozoa.

For the third week of fertility a highly significant breed effect was shown with the S.C.W.L. being superior regardless of the route or number of spermatozoa inseminated (Table 11).

Hatchability: Trial IA

During the first week there was a significant difference in hatchability between the neat and 1:1 diluted semen (Table 12). This indicated that neat semen provided higher hatchability regardless of the route of insemination. Within the diluted samples inseminated, there was a highly significant route effect on hatchability (Table 13) with the I.V.A.I. providing greater hatchability.

The second week showed no significant difference in hatchability from I.M.A.I. or I.V.A.I.

In the third week (Table 14) of hatchability, data indicated a highly significant breed and number effect with the S.C.W.L. breed being superior to the B.P.R.

Duration of Fertility: Trial IA

Duration of fertility showed two breed interactions. The first was a breed and number interaction (Table 15) indicating the S.C.W.L. to be superior at 12.5 million, 25 million and 100 million spermatozoa inseminated. When 50 million spermatozoa were inseminated, the B.P.R. was superior.

Secondly, the breed and route interaction (Table 16) indicated the

I.M.A.I. provided the longest duration of fertility for the S.C.W.L. while the I.V.A.I. provided longest duration of fertility for the B.P.R.

Trial IB

Fertility: Trial IB

The first week of fertility showed a significant breed and route interaction (Table 17). The S.C.W.L. had higher percent fertility with the I.V.A.I. while the B.P.R. had higher percent fertility with the I.M.A.I.

During the second week a significant route and number interaction with inseminations of neat semen was noted (Table 18). The I.V.A.I. provided greater fertility than I.M.A.I. at the 100 million spermatozoa level, while the 50 million level I.V.A.I. gave higher fertility than the 100 million I.V.A.I.

The second week of fertility was significantly better with the I.M.A.I. regardless of number or breed (Table 19).

The third week of fertility showed a significant breed effect with the S.C.W.L. being superior to the B.P.R. regardless of number or route of insemination (Table 20). A significant linear trend in fertility utilizing B.P.R. semen was shown with the 100 million level of spermatozoa inseminated being highest in fertility (Table 26).

Hatchability: Trial IB

Hatchability for the first week, within neat samples, showed a significant route and number interaction. One hundred million spermatozoa inseminated I.V. provided greater hatchability than 100 million level I.M.A.I. (Table 21), while the 50 million neat level I.V.A.I. was intermediate.

Within the diluted samples was found a significant breed effect (Table 22) indicating the B.P.R. to have greater hatchability regardless of number or route of insemination.

During the third week, hatchability showed a linear trend for the B.P.R. when I.M.A.I. was utilized with the 100 million level having greater hatchability for the neat and diluted samples (Table 23).

Duration of Fertility: Trial IB

There appeared to be a trend with the neat semen giving a longer duration of fertility of 17.81 days while the diluted semen gave a duration of fertility of 15-19 days. Also with the neat semen, there was a significant route and number interaction in which the I.M.A.I. gave the longest duration of fertility while the I.V.A.I. 100 million was the shortest in duration of fertility.

Within the diluted semen, there was a significant breed effect, highly significant breed and route interaction, and significant route interaction.

The S.C.W.L. gave the longest duration of fertility at 50 million, and the shortest duration of fertility at 100 million level diluted 1:1 (Table 24).

The B.P.R., although shorter in duration, showed a linear trend with the 100 million level being longest in duration (Table 24).

The I.M.A.I. route was significantly longer in duration of fertility than the I.V.A.I. (Table 25).

Trial IC

Fertility: Trial IC

During the first week was shown a highly significant interaction of route and number (Table 27). The I.M.A.I. gave highest fertility at 12.5, 25 and 50 million spermatozoa levels with 100 million being the lowest in fertility. The I.V.A.I. provided highest fertility at the 50 million level and lowest at the 12.5 million level of viable spermatozoa inseminated.

Fertility during the second week showed a significant breed and route effect. The breed and route interaction (Table 28) indicated that the S.C.W.L. were superior to the B.P.R. and the I.M.A.I. route was better than I.V.A.I.

The third week also indicated the highly significant fertility of the S.C.W.L. (Table 29).

Hatchability: Trial IC

During the second week, there was a route and number interaction (Table 30) within the diluted semen inseminations. The I.M.A.I. hatchability was high for the 12.5, 25 and 50 million spermatozoa levels, while the 100 million was the lowest level for the I.M.A.I.

For the third week, a highly significant breed effect was shown with the S.C.W.L. being double the percent hatchability of the B.P.R. (Table 29).

Duration of Fertility: Trial IC

The duration of fertility for the third trial showed a significant

effect from breed, route of insemination and number inseminated. The breed and route interaction (Table 31) showed the superiority of the I.M.A.I. for both breeds with the S.C.W.L. having longer duration of fertility than the B.P.R. Also there was a linear trend with the 100 million giving the longest duration of fertility (Table 32).

Experiment II

Trial IIA

Fertility: Trial IIA

Data from Trial IIA during the first week of fertility showed a significant route and dilution interaction (Table 35). The I.M.A.I. had a definite linear trend as the dilution decreased with the neat samples having the highest fertility and the 1:4 dilution having the lowest fertility. The I.V.A.I. showed the opposite linear trend with the neat semen having the lowest fertility.

During the second week of fertility, a highly significant route effect was evident (Table 36) along with a significant number effect (Table 37).

The route effect indicated the increased fertility utilizing the I.M.A.I. regardless of the dilution or number. The number effect indicated a linear trend during the second week with 100 million spermatozoa giving the highest fertility.

The fertility during the third week gave almost identical results for the number effect with only slightly smaller values (Table 38).

Hatchability: Trial IIA

During the first week, a significant route and number interaction was seen (Table 39). With the I.M.A.I. a trend indicated that as the

number of spermatozoa increased, the hatchability decreased. Just the opposite was true with the I.V.A.I. A definite linear trend indicated that as the number of spermatozoa increased, so did the hatchability increase.

During the second week and third week (Tables 40, 41, respectively), a significant dilution effect was evident indicating a better hatchability with 1:1 and 1:4 diluted samples.

Duration of Fertility: Trial IIA

Data analyzed for Trial IIA showed three major effects. (1) The route effect was highly significant (Table 42) and pointed out the increased duration of seven days utilizing the I.M.A.I. (2) A highly significant number effect was noted with a linear trend of duration when increased numbers of spermatozoa were inseminated (Table 43). The 50 million level appeared to be largest in overall duration regardless of route of insemination. (3) The significant dilution effect (Table 44) suggested the diluted samples gave slightly longer duration of two days with no difference between the 1:1 and 1:4 diluted samples.

Trial IIB

Fertility: Trial IIB

The first week of fertility had a significant route and number interaction (Table 45). The I.M.A.I. had a linear trend with decreasing fertility as the numbers of spermatozoa increased from 12.5 million spermatozoa to 100 million spermatozoa. Just the opposite occurred with the I.V.A.I. The fertility increased as the numbers of spermatozoa inseminated increased from 12.5 million to the 50 million level. There was a

slight drop in fertility at the 100 million level of spermatozoa inseminated.

The second week of fertility showed three major effects. (1) The route effect was highly significant (Table 46) which indicated the I.M. A.I. route as giving the higher fertility. (2) A significant number effect (Table 47) showed 100 million as being better than the 12.5 million, 25 million and 50 million levels of spermatozoa inseminated, regardless of route used and dilution used. A significant route and dilution interaction showed a reverse trend between the I.M.A.I. and I.V.A.I. (Table 48). With the I.M.A.I. a lower fertility was seen as the sample was more diluted. With the I.V.A.I. fertility increased as the sample was more diluted.

Hatchability: Trial IIB

The highly significant data on hatchability for the first week (Table 49) showed the I.V.A.I. route providing greater hatchability than the I.M.A.I.

The second week of hatchability reversed this trend to a significant degree with the I.M.A.I. providing greater hatchability than the I.V.A.I. (Table 50).

Duration of Fertility: Trial IIB

There were two main effects concerning duration of fertility in Trial IIB. (1) The highly significant route effect (Table 51) again pointed out the increased duration of six days utilizing I.M.A.I. (2) The significant number effect (Table 52) indicated the increased duration of fertility with increased numbers of spermatozoa inseminated, regardless of dilution of spermatozoa or route of insemination.

Experiment III

Trial IIIA

Fertility, Hatchability and Duration of Fertility

The 75 million level of viable spermatozoa inseminated was superior during Trial III as follows: (1) fertility during the first week (Table 56); (2) fertility during the second week (Table 57); (3) hatchability during the second week (Table 58); (4) overall duration of fertility (Table 59).

First Breed - First Week

By looking at the ratios of chicks hatched from the first breed inseminated during the first week, a significant breed effect was seen along with highly significant numbers, interval and breed-interval interactions. Also, a significant breed-number-interval interaction was seen (Table 60).

Breed Effect - When N.H. spermatozoa was used as the second breed inseminated, regardless of the first breed, the first breed had the highest ratio of chicks. No consistent correlation could be found concerning the second breed inseminated and the lower ratio of first breed chicks.

Number Effect - The 25 million level of insemination gave the highest ratios for the first breed chicks at all breed combinations except the S.C.W.L.-N.H. and B.P.R.-S.C.W.L. in which the 75 million level gave the highest ratio during the first week. Within the 75 million level, the B.P.R.-S.C.W.L. combination showed the highest ratio for the B.P.R. at both the one hour interval and the twenty-four hour interval of insemination. The one hour interval showed the highest ratio of the first breed chicks, regardless of number of spermatozoa inseminated.

First Breed - Second Week

During the second week a significant breed effect along with a significant breed-number-interval interaction was noted (Table 61). When looking at the chick ratios of the first breed inseminated, the data indicated the highest ratio of first breed S.C.W.L. chicks occurred when followed by N.H. as the second breed, regardless of the interval or number of spermatozoa inseminated. The lowest number of first breed chicks occurred when the S.C.W.L. were followed by the B.P.R.

First Breed - Total Trial IIIA

The ratio of first breed chicks for the total length of the trial showed a significant breed and number effect along with a significant interval effect.

Breed Effect - Overall ratios indicated more first breed chicks were sired by the S.C.W.L. and B.P.R. when followed by a second insemination of N.H. spermatozoa (Table 62), regardless of number or interval.

Number Effect - More of the first breed chicks, regardless of sequence of breed, were obtained with the lower number of spermatozoa - 25 million per insemination (Table 63).

Interval Effect - Over the total trial more first breed chicks occurred with the one hour interval (Table 64), regardless of the number of spermatozoa or breed inseminated.

Second Breed - First Week

During the first week the ratio of the second breed inseminated (Table 65) was as follows: (1) breed, significant; (2) number, highly significant; (3) interval, highly significant; (4) breed and interval

interaction, significant; (5) breed-interval-number interaction, significant.

(1) Breed - The highest ratio during the first week for the second breed inseminated was seen when the second breed was the S.C.W.L. or B.P.R. Conversely, the lowest ratios occurred when the N.H. was used as the second breed.

(2) Number - The higher number inseminated, 75 million, elicited the larger ratios of second breed chicks during the first week. Within the 75 million level the S.C.W.L. was good as the second breed, while the N.H. did poorly as the second breed of the one hour interval, but improved at the twenty-four hour interval.

(3) Interval - The twenty-four hour interval showed the highest ratio of second breed chicks.

Second Breed - Second Week

During the second week the ratios of the second breed chicks showed a significant breed effect along with a significant breed-number-interval interaction (Table 66). The breed effect showed the lowest ratio of second breed chicks when the second breed sire was the N.H., regardless of the first breed. The higher ratio was the S.C.W.L. inseminated as the first breed and the B.P.R. as the second breed inseminated.

Second Breed - Total Trial IIIA

The combined data of the second breed ratios for the entire trial showed a significant breed effect, number effect, a highly significant interval effect and a significant breed-interval interaction (Table 67).

When the N.H. spermatozoa was used as the second sire, the ratio of

their offspring was lower, regardless of the number of spermatozoa inseminated. The twenty-four hour interval of insemination showed a higher ratio of chicks hatched from the second breed than the one hour interval of insemination.

The 75 million level of insemination gave a higher ratio of second breed chicks than the 25 million level (Table 68).

Overall, the twenty-four hour interval showed more second breed chicks than the one hour interval (Table 67).

Duration of Fertility

The number of spermatozoa inseminated was significant in that 75 million spermatozoa showed a longer duration of fertility than 25 million spermatozoa inseminated (Table 69).

Trial IIIB

Fertility, Hatchability and Duration of Fertility

Fertility data in Trial IIIB indicated that the 75 million level of spermatozoa inseminated was superior during the first week (Table 70) and second week (Table 71).

Hatchability data showed a highly significant breed effect (Table 72) and a significant number effect. When the N.H. semen was used as the second breed, the hatchability was lowest during the second week, whereas the highest hatchability occurred when S.C.W.L. semen was inseminated as the second breed. Inseminations with 75 million spermatozoa showed the highest hatchability, regardless of insemination sequence (Table 73).

Duration of fertility showed a highly significant breed effect along with a significant breed-interval interaction (Table 74).

The number effect pointed out the longer duration of fertility with 75 million spermatozoa inseminated (Table 75).

The breed-interval interaction suggested the twenty-four hour interval was superior in duration of fertility for all breed combinations, except those which involved the N.H. and B.P.R. together (Table 74).

First Breed - First Week

During the first week, the ratios of the first breed inseminated indicated a significant breed effect (Table 76). It appeared that when the S.C.W.L. was used as the second breed, the first breed chick ratios were low. Also, when the N.H. was used as the second breed inseminated, the first week ratios were high for both the S.C.W.L. and B.P.R. as first breed inseminations.

First Breed - Second Week

The first breed ratios for the second week indicated a highly significant breed effect, number effect and a significant breed-number interaction (Table 77).

When the N.H. was used as the first breed inseminated, the ratio of N.H. chicks hatched was lowest for the second week. When N.H. spermatozoa was used as the second breed, the ratios of chicks hatched for first breeds (S.C.W.L. or B.P.R.) inseminated were higher.

The ratio of the first breed chicks during the second week was highest with 75 million spermatozoa inseminated.

First Breed - Total Trial IIIB

The ratios of first breed chicks for the total experiment had a highly significant breed effect and a significant breed-interval interaction (Table 78). During the first week the first breed which showed the lowest number of chicks was the N.H., particularly when followed by the S.C.W.L. as the second breed inseminated at both the one hour and twenty-four hour intervals.

If N.H. spermatozoa was used as the second breed inseminated, the result was more first breed chicks at both the one hour and twenty-four hour intervals during the first week.

Second Breed - First Week

The first week ratios for the second breed inseminated showed a highly significant breed effect (Table 79) and a significant number-interval interaction (Table 80).

The S.C.W.L. was the highest while the N.H. showed the lowest ratios as the second breed inseminated.

The one hour interval with 25 million spermatozoa inseminated gave the highest number of second breed chicks during the first week.

Second Breed - Second Week

The second week ratios for the second breed inseminated had a highly significant breed effect, number effect and a significant breed-number interaction (Table 81). When the S.C.W.L. spermatozoa was used as the second breed inseminated, the ratios were highest at the 25 million level of spermatozoa inseminated. The N.H. spermatozoa used as the second breed inseminated gave low ratios at both 25 million and 75 million levels of

spermatozoa inseminated. The best for both levels of 25 million and 75 million spermatozoa inseminated was the N.H. followed by the S.C.W.L. Within the 75 million level of insemination, the N.H. followed by the B.P.R. gave the lowest ratio of chicks hatched.

The 25 million level gave the highest number of second breed chicks during the second week compared to the lower number of chicks at the 75 million level (Table 81).

Second Breed - Total Trial IIIB

Overall, the second breed showed a highly significant breed effect and significant number-interval interaction (Tables 82 and 83).

When N.H. was used as the second breed inseminated, the ratios were lowest, regardless of what the first breed inseminated was. When the S.C.W.L. was used as the second breed inseminated, the ratios were highest.

The ratio of the second breed chicks was higher at the one hour interval of insemination particularly within the 25 million level of spermatozoa inseminated.

Duration of Fertility

The duration of fertility for the overall experiment, showed a significant breed effect, highly significant number effect (Table 84) and a significant breed-interval interaction (Table 85).

When the S.C.W.L. was the second breed inseminated, the duration of fertility was longest. Duration of fertility was shortest when the N.H. was the second breed inseminated.

The 75 million level of insemination gave the longest duration of fertility, regardless of the breed or interval.

When the S.C.W.L. was followed by an N.H. insemination one hour later, duration of fertility was very short; but duration of fertility was longer with the same breed sequence at the twenty-four hour interval.

Trial IIIC

Fertility, Hatchability and Duration of Fertility

During both the first and second weeks (Tables 86 and 87), the fertility was significantly higher with 75 million spermatozoa inseminated.

Duration of fertility for the second breed inseminated was highly significant indicating the S.C.W.L. as the breed giving the longest duration of fertility and the N.H. having the shortest duration of fertility (Table 88).

Duration of fertility for the third breed inseminated was significant having the same trend seen for the second breed inseminated. The S.C.W.L. had the longest duration of fertility when utilized as the third breed; B.P.R. was intermediate, and N.H. had the shortest duration of fertility when utilized as the third breed inseminated.

The ratios of offspring sired by the second breed inseminated during the first week of fertility showed a highly significant breed effect.

Second Breed - First Week

When the S.C.W.L. spermatozoa was used as the second breed inseminated (twenty-four hours after the first insemination), there was a greater number of S.C.W.L. chicks in the first week of fertility than either B.P.R. or N.H. chicks. The B.P.R. was intermediate, and the N.H. was lowest in number of chicks produced.

Third Breed - First Week

Also during the first week of fertility the ratios of chicks hatched from the third breed inseminated were highly significant. Again the S.C.W.L. ratio of chicks hatched was highest with the N.H. ratio of chicks hatched being lowest.

Second Breed - Second Week

During the second week of fertility, the ratios of chicks hatched for both the second and third breed inseminated were highly significant, indicating the S.C.W.L. as being superior to the B.P.R. and N.H., respectively.

Second and Third Breed - Total Trial IIIC

The ratios for the overall trial indicated the S.C.W.L. as being superior when inseminated either as the second or third breed. The B.P.R. was intermediate as the second or third breed inseminated. The N.H. had the lowest ratio of chicks hatched when the N.H. males were used for either the second or third breed inseminated.

Trial IIID

Fertility, Hatchability and Duration of Fertility

The experimental design of Trial IIID was identical to Trial IIIC (Table 55).

Fertility: Trial IIID

Percent fertility showed a highly significant number effect for the second week (Table 89), which indicated the 75 million level of spermatozoa

as being superior to the 25 million level of spermatozoa inseminated.

Duration of Fertility

The first breed inseminated showed a significant number effect with 75 million having the longest duration of fertility of 5.8 days compared to the lower duration of 3.75 days for the 25 million level of spermatozoa inseminated (Table 90). It should be pointed out that duration of fertility of the individual breeds was calculated from the day of insemination of that particular breed.

The breed effect was highly significant for the duration of fertility of the first breed inseminated (Table 91). The N.H. showed the shortest duration of fertility while the S.C.W.L. and B.P.R. were longest.

The second breed inseminated had a highly significant number effect (Table 92) indicating that the 75 million level was better for the second breed inseminated.

The total duration of fertility of Trial IIID (Table 93) showed the 75 million level of spermatozoa inseminated being superior to the 25 million level. This number effect was highly significant.

First Breed - Second Week

Ratios of chicks hatched from the first breed inseminated showed a highly significant breed effect during the second week of Trial IIID (Table 94). When N.H. spermatozoa was the first breed inseminated, the smaller number of chicks sired during the second week was N.H.

Third Breed - Second Week

For the second week, the ratios of chicks hatched for the third breed inseminated indicated the B.P.R. as being superior with the N.H. having the lowest ratios (Table 95).

DISCUSSION

Experiment I

Trial IA

Fertility: Trial IA

Trial IA was the first of three trials. An outline of the experimental design and analysis of variance may be seen in Tables 1 and 4, respectively.

The breed effect indicated the superiority of the S.C.W.L. spermatozoa on fertility during the first and third weeks. S.C.W.L. hens had a higher percent fertility when mated with S.C.W.L. males as compared to the B.P.R. male and S.C.W.L. female cross. Perhaps this higher fertility is due to some physiological or anatomical factor which enhances the S.C.W.L. female predilection for S.C.W.L. spermatozoa or discrimination toward B.P.R. spermatozoa.

Of more importance was the effect of sperm cell numbers and the route of insemination on the fertility, hatchability and duration of fertility, and the possible relationship to sperm storage areas, the uterovaginal host glands and the infundibular crypts.

As seen in the linear trend in Table 8, during the first week of fertility, the uterovaginal junction had the ability to hold and support up to 100 million spermatozoa diluted 1:1. At the same time, the infundibular crypts (I.M.A.I.) appeared to reach their maximum potential with 50 million spermatozoa diluted 1:1.

The first week may be the most critical when relating capacity of sperm cell storage areas to numbers of spermatozoa inseminated because of the movement of the spermatozoa throughout the oviduct. In other words, can we safely assume that spermatozoa from the I.M.A.I. were actually responsible for the fertility, hatchability and duration associated with that insemination? Or had some of the spermatozoa migrated to the host glands where they were released for fertilization? This author assumed that transfer of spermatozoa from one storage site to another was negligible at best (Bobr et al., 1964b).

During the second week of fertility, there was no difference between the neat and diluted samples when the 50 million and 100 million I.V.A.I. were compared. This was as expected since Beltsville Extender has been found to have no effect on fertility, hatchability or duration of fertility as discussed in a personal communication with Dr. T. Sexton (1977).

The second week showed a linear trend (Table 10) for the I.V.A.I. indicating the capability of the host glands located at the uterovaginal junction to adequately hold 100 million spermatozoa I.V.A.I. Conversely, the infundibular crypts following I.M.A.I. seemed to store the 50 million spermatozoa best with the highest fertility. The 100 million level appeared to overwhelm the crypts thus a lower fertility.

The third week of fertility (Table 11) completed the highly significant pattern of S.C.W.L. spermatozoa being superior to the B.P.R. spermatozoa when inseminated in the S.C.W.L. regardless of route, I.M.A.I. or I.V.A.I., and number, 12.5 million, 25 million, 50 million or 100 million.

Hatchability: Trial IA

During the first week, the neat samples resulted in a higher hatchability regardless of the route of insemination used. This was most likely

due to the increased numbers of birds inseminated with diluted semen (Table 1). Within the diluted samples, the intravaginal artificial insemination gave the higher hatchability than I.M.A.I. regardless of the number of spermatozoa inseminated. This seemed logical if the U.V.J. host glands had the ability to "filter out" abnormal spermatozoa. Theoretically only viable spermatozoa were released for fertilizing past the U.V. junction.

The opposite situation held for the infundibular crypts which evidently did not have the ability to segregate the normal from the abnormal spermatozoa. Thus a higher number of spermatozoa which were incomplete or having poor fertilizing capabilities may have been released resulting in poor hatchability.

During the third week of hatchability the S.C.W.L. had greater hatchability than B.P.R. regardless of the route of insemination. This further added to the breed effect seen in fertility and duration of fertility. Possibly a reaction by the S.C.W.L. female limited the B.P.R. spermatozoa activity. Also there may have been some chemical factor within the S.C.W.L. female which limited or retarded the B.P.R. spermatozoa's capabilities particularly at the lower numbers (Table 14).

Duration of Fertility: Trial IA

There was no significant difference in hatchability due to the use of Beltsville Extender when used to dilute the samples.

The tendency for the S.C.W.L. to be superior and elicit a longer duration of fertility was seen in this experiment (Table 15). Also the I.M.A.I. was the best for the S.C.W.L., but not for the B.P.R. This could be accounted for by referring to the raw data. Several of the hens

inseminated with B.P.R. spermatozoa I.M.A.I. failed to return to production. Because the number of females remained constant, any that did not return to production were included in the data thus lowering duration for that group. Normally one would expect the I.M.A.I. to have a longer duration than the I.V.A.I. (Ogasawara et al., 1969).

Trial IB

Before discussing Trial IB, several points should be made. (1) The males were the same males used for Trials IA and IC. (2) The hens were determined to be infertile before insemination. (3) The technique for insemination was basically the same although improvements in technique for accuracy of numbers were made.

The experimental design (Tables 2 and 5) was identical with Experiment I, Trials IA and IC for the 1:1 dilution, but for further comparison, the level of 100 million neat spermatozoa was added to the I.M.A.I. route for both S.C.W.L. and B.P.R. spermatozoa.

Fertility: Trial IB

Fertility for the first week showed an unusually low fertility for the S.C.W.L. when I.M.A.I. was used creating the breed and route interaction (Table 17). This was due to a significantly large number of birds which failed to lay eggs during the first week but returned to production with fertile eggs during the second week of the trial. Thus the fertility was lowered for the first week.

This trend was not significant during the second week (Table 19) of fertility because of the return to production of fertile eggs. Also the I.M.A.I. became highly significant regardless if either S.C.W.L. or B.P.R.

spermatozoa was used. How this might be related to the storage sites may be quite complicated or might be a simple matter of anatomical relationships of storage site to the site of fertilization. The storage site for I.M.A.I. is the infundibular crypts which are located adjacent to the site of fertilization providing little difficulty or distance in terms of spermatozoa travel.

Remembering, of course, that only the I.M.A.I. gave a third week's fertility data, the S.C.W.L. had significantly higher fertility than the B.P.R. during the third week of fertility (Table 20). With the B.P.R. semen, the neat samples at the 100 million level had a fertilizing capacity greater than the 1:1 dilution of 100 million spermatozoa (Table 26). Very possibly the extra volume added by the diluent had some effect on the spermatozoa's ability to fertilize during the third week. It could be that more spermatozoa, for example, 200 million 1:1, could elicit fertility equal to 100 million neat.

Duration of Fertility: Trial IB

The mean fertility for diluted samples inseminated was slightly lower than with neat samples. This occurred because the extra levels (12.5 and 25 million) of spermatozoa were included into the data analysis for diluted samples and not the neat samples.

The I.M.A.I. route through the infundibular crypts gave the longest duration for both neat and diluted samples.

Unlike Trial IA, a definite linear trend was obvious (Table 24) for the B.P.R. samples diluted 1:1 with 100 million level of insemination having the longest duration of fertility. The linear trend was lacking with the S.C.W.L. samples due to a peaking in duration of fertility at

50 million along with the lowest duration of fertility at 100 million. This was most likely related to the filling and storage potentials of the S.C.W.L. host glands and infundibular crypts, discussed earlier along with their association to breeds.

Trial IC

Fertility: Trial IC

The experimental design (Tables 3 and 6) was identical to Experiment II except for the deletion of 50 million I.V.A.I. for both breeds. The techniques were similar.

Fertility the first week of Trial IC was almost identical to Trial IA. Compare Tables 7 and 27. The I.M.A.I. for the 12.5 million and 25 million was consistent for both trials, while a peak fertility was reached near the 50 million mark with a decline from 50 million to 100 million. In both cases the infundibular crypts appeared to be most functional when inseminated with 50 million spermatozoa diluted 1:1. Whereas data from inseminations of up to 100 million spermatozoa into the U.V. junction appeared to be linear in trend.

The second week in Trial IC followed the same trend (Table 28) with the S.C.W.L. being superior and the I.M.A.I. dominating over the I.V.A.I. The third week (Table 29) confirmed week two data with S.C.W.L. spermatozoa inseminated I.M.A.I. as the best combination.

Experiment II

Trials IIA-IIB

Because of the similarity, both Trials IIA and IIB will be discussed together. The experimental design (Table 33) and the analysis of variance (Table 34) were identical for both trials.

The overall fertility in both trials indicated the I.M.A.I. route as being superior, regardless of the number of spermatozoa inseminated or dilution of sample.

The trend for the I.M.A.I. indicated a higher fertility during the first week utilizing 12.5 million and 25 million spermatozoa inseminated (Table 45). Evidently these low numbers were compatible with the storage area available in the infundibular crypts. Higher numbers of spermatozoa may have overwhelmed the crypts creating lower fertility during the first week. This trend in numbers was not maintained for the third week of fertility. The higher numbers of spermatozoa inseminated showed higher fertility by the third week (Table 38).

The route and number interaction was not significant for weeks two and three in either trial. Therefore, the overloading of the storage area appeared to be only temporary. This corresponded with the lower hatchability during the first week (Table 49) utilizing I.M.A.I. As discussed in Experiment I, the increased numbers of abnormal spermatozoa available for potential fertilization may have lowered the hatchability during the first week. This may have been the case (Table 49) for hatchability the first week which indicated lower hatchability with I.M.A.I. than during the second week (Table 50). Overall fertility, hatchability and duration of fertility during both trials showed the superiority of

the I.M.A.I. This supported the work of Van Krey et al. (1964) and Ogasawara and Lorenz (1966) that increased abnormal spermatozoa reaching the infundibular crypts with I.M.A.I. resulted in increased potential for decreased hatchability during the first week of the trials. This held true if, in fact, the U.V.J. host glands filtered out abnormal spermatozoa and released basically only normal viable spermatozoa.

The superiority of the I.M.A.I. could also be seen with the longer duration of fertility utilizing I.M.A.I. (Tables 42 and 51). This corresponded with Van Krey et al. (1964) and Experiment I.

The linear trend also was consistent with Experiment I in that the lower numbers gave slightly shorter duration of fertility and the higher numbers gave greater duration of fertility (Tables 43 and 52). The actual spread of the overall means of duration of fertility was not as great as first expected because of the higher duration of fertility at the lower numbers utilizing I.M.A.I.

During the first trial the dilution effect was significant for hatchability during the second week and duration of fertility during the entire trial (Tables 40 and 44) which was indicated by better hatchability and longer duration of fertility with the 1:1 and 1:4 dilution. The extender and technique used were the same as in Trial IIB, which showed no dilution effect. One possible explanation involved the concept of virgin hens. Trial IIA was done on hens which, although mature, had never been inseminated either I.M.A.I. or I.V.A.I. This virgin condition could have played some physiological role in the dilution effect. The trend might have been related to the virgin condition of the uterovaginal host glands, (Trial IIA) or the used condition of the host glands (Trial IIB).

By comparing the above data in Tables 40 and 44 to Table 35, and

looking at just I.V.A.I., it could be noted that there was a similarity in values with the neat samples being lowest. This was opposite of the I.M.A.I. (Table 35) which suggested the diluent had a detrimental action on fertility during the first week by possibly over filling the infundibular crypts. It was just possible that the effect of extender was to aid in dispersion of the spermatozoa through parts of the host glands which cannot be reached by neat semen. There was no significant dilution and number interaction in either Trials IIA or IIB.

Experiment III

Trials IIIA and IIIB

Trials IIIA and IIIB were exactly the same in experimental design (Table 53). All N.H. hens were in production and had infertile eggs prior to insemination. The three different breeds of males utilized (N.H., S.C.W.L. and B.P.R.) were handled at the same intervals prior to and during the semen collection.

During the first week of fertility, which began on the third day following the first insemination, the breed effect was significant for the first breed inseminated. The data indicated the superiority or dominant influence of the S.C.W.L. spermatozoa in the second insemination over the N.H. and B.P.R. semen from the first insemination (Tables 60 and 76). This data appeared to be consistent throughout both Trials IIIA and IIIB. An attempt was made to rationalize this superior effect of the S.C.W.L. based on number of spermatozoa inseminated, but careful examination of the insemination records indicated the equalized numbers of spermatozoa were, in fact, equal (see Method and Materials). Any breed effect

was due to possible interaction of the different breeds of semen or some hen reaction to the semen. Because all inseminations were intravaginal, the role of the host glands may have been related to the breed effect, either directly or indirectly.

A second aspect of the ratios of chicks hatched during the entire length of both Trial IIIA and Trial IIIB was the low numbers of N.H. chicks which hatched. This occurred both when N.H. spermatozoa was used as the first breed inseminated or the second breed inseminated. As a precautionary measure, a pre and post trial experiment was performed to determine the fertility, hatchability and duration of fertility of the three different breeds when crossed with the N.H. hens.

When the N.H. males were crossed with the N.H. females, similar results were found compared to the B.P.R. and S.C.W.L. matings with N.H. females. This ruled out the possibility of N.H. males being inferior to the other two breeds. Therefore, it could be assumed that the low number of chicks sired by the N.H. males was due either to the spermatozoa interaction, seminal fluid interaction, hen and semen interaction or some influence of the storage sites of the hen.

The ratios of chicks hatched for the first breed inseminated over the total time was similar for both trials and may give some insight as to the introduction, release and transport of spermatozoa to the site of fertilization (Tables 62, 63, 64 and 78). During the first trial, more chicks were sired by the first breed inseminated at the one hour interval (Table 64) indicating the storage sites were more receptive to the first breed when the second insemination was only one hour later. Perhaps the shorter interval allowed for the U.V.J. host glands to regulate the order of storage and release of spermatozoa while the passage of the egg or

oviposition which occurred within the twenty-four interval altered this release of spermatozoa.

During Trial IIIA the lower number of spermatozoa inseminated (25 million) was also favorable to the first breed inseminated (Table 63). This may indicate that the U.V. junction storage site may regulate the first breed introduced as the first breed released, at the lower number of spermatozoa. Whereas the higher number of spermatozoa (75 million) may overwhelm the U.V. junction and its ability to release spermatozoa in the same sequence as it was introduced.

The second breed inseminated sired the highest number of chicks at the 75 million level (Tables 63 and 68) at the twenty-four hour interval.

In both trials, the duration of fertility was higher at the 75 million level of insemination (Tables 69 and 84). No significant difference in duration of fertility was noted between the one hour interval and the twenty-four hour interval within each trial although the overall duration of fertility was longer in Trial IIIA. (Compare Tables 69 and 84). Because the spermatozoa numbers were equalized after estimation of numbers, this difference was most likely due to some environmental or management stress on the birds and not due to lack of or decreased numbers of spermatozoa.

Trials IIIC and IIID

Both Trials IIIC and IIID had the same experimental design (Table 55). The males and females used were the same for each trial. The semen was collected, tested and inseminated in the same way. The only factors that may possibly have altered the results were those in managment which

were minimal.

Special care was taken when collecting semen to use those males which had not been milked the previous day. This would hopefully cut down on the number of immature spermatozoa in the sample.

One factor which may have affected the results was the slight decrease in egg production during the start of each trial. This was believed to be due to the stress of handling the hens three days in a row.

In both trials, the 75 million level of spermatozoa inseminated was superior for (1) fertility (Tables 86, 87 and 89) and (2) duration of fertility (Tables 90, 92 and 93). Although the 75 million level of insemination was not significantly longer for duration of fertility in Trial IIIA, the 75 million level of spermatozoa gave a longer duration of fertility for every breed combination and total duration of fertility.

The duration of fertility for the first breed inseminated (Table 90) was quite low at both the 25 and 75 million level. This may be due to the masking effect of the second and third inseminations.

When the duration of fertility of the first breed inseminated in Trials IIIA and IIIB was compared to the first breed inseminated in Trials IIIC and IIID, the duration of fertility was longer in Trials IIIA and IIIB. Possibly the third insemination in Trials IIIC and IIID had some deleterious effect thereby decreasing the ability of the first breed inseminated to maintain its fertilizing capacity.

Another explanation involved the overwhelming of the uterovaginal host glands with the third insemination. If as previously indicated, 100 million spermatozoa appeared to elicit the longest duration, then the additional insemination particularly at the 75 million level might mask the ability of the host glands to regulate the release of the spermatozoa

as inseminated. This resulted in the first and second breed duration of fertility as being shorter (Tables 90 and 92) when compared to the overall duration of fertility (Table 93) of Trial IIID and Trials IIIA and IIIB.

Table 1

EXPERIMENTAL DESIGN OF TRIAL IA[1]

Inseminations with Neat Semen[2]		Inseminations with Semen Diluted 1:1			
S.C.W.L.	B.P.R.	S.C.W.L.		B.P.R.	
I.V.- 50	I.V.- 50	I.V.- 12.5	I.M.- 12.5	I.V.- 12.5	I.M.- 12.5
I.V.-100	I.V.-100	I.V.- 25	I.M.- 25	I.V.- 25	I.M.- 25
		I.V.- 50	I.M.- 50	I.V.- 50	I.M.- 50
		I.V.-100	I.M.-100	I.V.-100	I.M.-100

Table 2

EXPERIMENTAL DESIGN OF TRIAL IB[1]

Inseminations with Neat Semen[2]		Inseminations with Semen Diluted 1:1			
S.C.W.L.	B.P.R.	S.C.W.L.		B.P.R.	
		I.V.- 12.5	I.M.- 12.5	I.V.- 12.5	I.M.- 12.5
I.M.-100	I.M.-100	I.V.- 25	I.M.- 25	I.V.- 25	I.M.- 25
I.V.- 50	I.V.- 50	I.V.- 50	I.M.- 50	I.V.- 50	I.M.- 50
I.V.-100	I.V.-100	I.V.-100	I.M.-100	I.V.-100	I.M.-100

Table 3

EXPERIMENTAL DESIGN OF TRIAL IC[1]

Inseminations with Neat Semen[2]		Inseminations with Semen Diluted 1:1			
S.C.W.L.	B.P.R.	S.C.W.L.		B.P.R.	
		I.V.- 12.5	I.M.- 12.5	I.V.- 12.5	I.M.- 12.5
		I.V.- 25	I.M.- 25	I.V.- 25	I.M.- 25
I.M.-100	I.M.-100	I.V.- 50	I.M.- 50	I.V.- 50	I.M.- 50
I.V.-100	I.V.-100	I.V.-100	I.M.-100	I.V.-100	I.M.-100

[1]Eight Single Comb White Leghorn (S.C.W.L.) females per group were artificially inseminated intramagnally (I.M.) or intravaginally (I.V.) with 12.5 million (12.5), 25 million (25), 50 million (50), or 100 million (100) viable spermatozoa.

[2]Semen was collected from Single Comb White Leghorn (S.C.W.L.) or Barred Plymouth Rock (B.P.R.) males and inseminated I.M. or I.V. fresh as pure semen (neat), or diluted with Beltsville Extender (1:1).

Table 4

ANALYSIS OF VARIANCE OF TRIAL IA

Source	D.F.	Mean Squares for Traits: Fertility Week - 1	Hatchability Week - 1	Fertility Week - 2	Hatchability Week - 2	Duration of Fertility
Neat vs diluted (1:1)	1	111.492	4,956.504*	1,111.474	434.716	5.720
Within Neat						
Breed (B)	1	253.526	454.542	65.853	1,491.118	16.0
Number of Sperm (N)	1	0.043	2.907	583.464	241.492	49.0
B x N	1	845.791	1,193.703	0.555	1,188.870	20.25
Within Diluted (1:1)						
Breed (B)	1	4,008.560*	2,582.130	1,840.126	182.960	121.5
Number of Sperm (N)	3	2,895.674*	1,560.610	2,083.296	1,823.626	53.736
B x N	3	1,554.408	135.508	743.287	1,015.091	89.083*
Route of Insemination (R)	1	3,310.207	16,115.738**	8,195.283**	3,143.046	38.521
B x R	1	1,814.004	3,116.319	8,054.771**	1,656.456	450.188**
N x R	3	2,683.603*	981.657	2,334.788*	1,417.145	17.451
B x N x R	3	462.490	781.063	2,108.528*	283.609	66.313
Residual or Error	92	970.502	929.370	768.229	1,122.609	32.701

* - Significant by one way analysis of variance. ($P<.05$)
** - Highly significant by one way analysis of variance. ($P<.01$)

Table 5

ANALYSIS OF VARIANCE OF TRIAL IB

Source	D.F.	Mean Squares for Traits: Fertility Week - 1	Hatchability Week - 1	Fertility Week - 2	Hatchability Week - 2	Duration of Fertility
Neat v. diluted (1:1)	1	307.862	75.482	1,294.116	44.881	164.065*
Within Neat						
Breed (B)	1	692.850	102.603	0.152	234.145	3.125
Route and Number (RN)	2	1,115.763	4,002.232*	3,503.170*	1,604.715	177.688*
B x RN	2	431.627	361.499	624.472	891.649	9.562
Within Diluted						
Breed (B)	1	1,527.452	4,774.401*	2,192.204	280.714	231.260*
Number of Sperm (N)	3	1,581.581	478.918	1,779.413	907.329	70.927
B x N	3	658.160	1,130.574	791.337	416.040	160.149**
Route of insemination (R)	1	1.637	3,570.458	10,180.498**	812.178	176.333*
B x R	1	5,643.630*	1,689.339	340.934	21.407	6.021
N x R	3	1,315.321	1,547.580	717.411	777.827	9.292
B x N x R	3	272.257	719.914	891.687	1,288.835	56.507
Residual or Error	106	967.917	961.411	856.585	1,190.769	36.738

* Probability less than .05
** Probability less than .01

Table 6

ANALYSIS OF VARIANCE OF TRIAL IC

Source	D.F.	Mean Squares for Traits: Fertility Week - 1	Hatchability Week - 1	Fertility Week - 2	Hatchability Week - 2	Duration of Fertility
Neat vs Diluted (1:1)	1	1,546.967	2,284.974	2.307	31.796	2.269
Within Neat						
Breed (B)	1	2,399.20	715.369	110.811	90.754	57.042
Route of Insemination (R)	1	3,668.703	2,548.439	406.585	2,621.676	123.521
B x R	1	383.635	250.939	2,764.278	715.026	130.021
Within Diluted (1:1)						
Breed (B)	1	1,645.484	3,354.871	17,117.905**	3,797.147	882.094**
Number of Sperm (N)	3	655.138	250.728	787.290	1,568.465	120.122*
B x N	3	1,960.827	762.148	370.388	481.132	3.955
R	1	37.879	2,729.254	9,205.126**	1,502.873	494.083**
B x R	1	2,100.263	698.259	5,745.360*	2,418.473	396.750**
N x R	3	4,604.380**	987.325	2,591.888	9,015.939**	59.347
B x N x R	3	1,211.040	2,119.286	850.363	1,510.275	17.764
Residual or Error	100	968.811	1,200.343	1,012.459	1,369.567	43.346

* Probability less than .05
** Probability less than .01

Table 7

BREED EFFECT* WITH THE SINGLE COMB WHITE LEGHORN (S.C.W.L.) AND BARRED PLYMOUTH ROCK (B.P.R.) SEMEN DURING WEEK-1 OF FERTILITY

Breed of Semen Inseminated	Mean Percent Fertility
S.C.W.L.	67.58
B.P.R.	54.66

Table 8

INTERACTION* BETWEEN ROUTE OF INSEMINATION (INTRAMAGNAL - I.M. OR INTRA-VAGINAL - I.V.) AND NUMBER OF SPERMATOZOA INSEMINATED DURING WEEK-1 OF FERTILITY

Number of Spermatozoa Inseminated (millions)	Mean Percent Fertility Route of Insemination	
	I.V.	I.M.
12.5	61.54a	62.95a
25	57.85a	62.74a
50	74.90ab	74.79ab
100	83.43b	69.85ab

*Probability less than .05

a,b - Statistical significance utilizing Tukey's Test (probability less than .05) is denoted by different letters in rows or columns. Example: By utilizing Tukey's Test, mean value differences greater than 17.35 (Table 8) are significant at the .05 level. Therefore, 61.54a is significantly different from 83.43b but not significantly different from 74.90ab. Subscript a is always significantly different from subscript b but not subscript ab.

Table 9

INTERACTION** BETWEEN SINGLE COMB WHITE LEGHORN (S.C.W.L.) SEMEN OR BARRED PLYMOUTH ROCK (B.P.R.) SEMEN AND THE ROUTE OF INSEMINATION (INTRAMAGNAL - I.M. OR INTRAVAGINAL - I.V.) DURING WEEK-2 OF FERTILITY

Breed of Semen Inseminated	Mean Percent Fertility I.V.	I.M.
S.C.W.L.	30.09a	69.12b
B.P.R.	47.25ab	47.41ab

Table 10

INTERACTION* BETWEEN ROUTE OF INSEMINATION (INTRAMAGNAL - I.M. OR INTRAVAGINAL - I.V.) AND NUMBER OF SPERMATOZOA INSEMINATED DURING WEEK -2 OF FERTILITY

Number of Spermatozoa Inseminated (millions)	Mean Percent Fertility I.V.	I.M.
12.5	31.77a	59.63b
25	34.79ab	55.38b
50	38.62ab	78.10c
100	49.50b	39.97a

*Probability less than .05
**Probability less than .01

a, b, c - Statistical significance utilizing Tukey's Test (probability less than .01 or .05, respectively) is denoted by different letters in rows or columns.

Table 11

BREED EFFECT** WITH SINGLE COMB WHITE LEGHORN (S.C.W.L.) AND BARRED PLYMOUTH ROCK (B.P.R.) SEMEN DURING WEEK-3 OF FERTILITY

Breed of Semen Inseminated	Mean Percent Fertility
S.C.W.L.	39.03
B.P.R.	17.03

Table 12

DILUTION EFFECT* WITH PURE SEMEN (NEAT) AND DILUTED SEMEN DURING WEEK-1 OF HATCHABILITY

Dilution of Semen Inseminated	Mean Percent Hatchability
Neat	75.61
Diluted[1]	61.18

Table 13

ROUTE EFFECT** WITHIN DILUTED SAMPLES WITH INTRAMAGNAL (I.M.)AND INTRA-VAGINAL (I.V.) ROUTES OF INSEMINATION DURING WEEK-1 OF HATCHABILITY

Route of Insemination	Mean Percent Hatchability
I.V.	74.92
I.M.	47.43

*Probability less than .05
**Probability less than .01

[1]Semen diluted 1:1 with Beltsville Extender.

Table 14

INTERACTION** DURING WEEK-3 OF HATCHABILITY BETWEEN SINGLE COMB WHITE LEGHORN (S.C.W.L.) OR BARRED PLYMOUTH ROCK (B.P.R.) SEMEN AND THE NUMBER OF SPERMATOZOA INSEMINATED

Number of Spermatozoa Inseminated (millions)	Mean Percent Hatchability S.C.W.L.	B.P.R.
12.5	41.68c	7.73ab
25	39.51c	3.32a
50	30.43bc	32.10c
100	44.50c	27.69bc

Table 15

INTERACTION* BETWEEN SINGLE COMB WHITE LEGHORN (S.C.W.L.) OR BARRED PLYMOUTH ROCK (B.P.R.) SEMEN AND THE NUMBER OF SPERMATOZOA INSEMINATED ON DURATION OF FERTILITY

Number of Spermatozoa Inseminated (millions)	Duration of Fertility (Days) S.C.W.L.	B.P.R.
12.5	17.17cd	13.42ab
25	13.33ab	12.42a
50	15.17abc	17.50cd
100	18.92d	12.25a

*Probability less than .05
**Probability less than .01

a,b,c,d - Statistical significance utilizing Tukey's Test (probability less than .05 or .01, respectively) is denoted by different letters in rows or columns.

Table 16

INTERACTION* BETWEEN SINGLE COMB WHITE LEGHORN (S.C.W.L.) OR BARRED PLYMOUTH ROCK (B.P.R.) SEMEN AND ROUTE OF INSEMINATION (INTRAMAGNAL - I.M. OR INTRAVAGINAL - I.V.) ON DURATION OF FERTILITY

Breed of Semen Inseminated	Duration of Fertility (Days)	
	Route	
	I.V.	I.M.
S.C.W.L.	12.19a	18.13b
B.P.R.	16.06ab	12.81a

Table 17

INTERACTION* DURING WEEK-1 OF FERTILITY BETWEEN SINGLE COMB WHITE LEGHORN (S.C.W.L.) OR BARRED PLYMOUTH ROCK (B.P.R.) SEMEN AND ROUTE OF INSEMINATION (INTRAMAGNAL - I.M. OR INTRAVAGINAL - I.V.)

Breed of Semen Inseminated	Mean Percent Fertility	
	Route	
	I.V.	I.M.
S.C.W.L.	68.88ab	52.34a
B.P.R.	55.17ab	71.16b

*Probability less than .05

a, b - Statistical significance utilizing Tukey's Test (probability less than .05) is denoted by different letters in rows or columns.

Table 18

INTERACTION* DURING WEEK-2 OF FERTILITY BETWEEN ROUTE OF INSEMINATION (INTRAMAGNAL - I.M. OR INTRAVAGINAL - I.V.) AND THE NUMBER OF SPERMATOZOA INSEMINATED

Number of Spermatozoa Inseminated (millions)	Mean Percent Fertility Route	
	I.V.	I.M.
50	78.53b	-----
100	66.83ab	-----
100	-----	58.17a

Table 19

ROUTE EFFECT** DURING WEEK-2 OF FERTILITY WITH INTRAMAGNAL (I.M.) AND INTRAVAGINAL (I.V.) ROUTE OF INSEMINATION

Route of Insemination	Mean Percent Fertility
I.V.	33.65
I.M.	55.50

Table 20

BREED EFFECT* WITH THE SINGLE COMB WHITE LEGHORN (S.C.W.L.) AND BARRED PLYMOUTH ROCK (B.P.R.) SEMEN DURING WEEK-3 OF FERTILITY

Breed of Semen Inseminated	Mean Percent Fertility
S.C.W.L.	46.62
B.P.R.	30.60

*Probability less than .05
**Probability less than .01

a, b - Statistical significance utilizing Tukey's Test (probability less than .05) is denoted by different letters in rows or columns.

Table 21

INTERACTION* DURING WEEK-1 OF HATCHABILITY BETWEEN INTRAMAGNAL (I.M.) OR INTRAVAGINAL (I.V.) ROUTES OF INSEMINATION AND NUMBER OF SPERMATOZOA INSEMINATED

Route of Insemination	Number of Semen Inseminated	Mean Percent Hatchability
I.V.	50 Neat	75.70b
I.V.	100 Neat	82.88b
I.M.	100 Neat	48.06a

Table 22

BREED EFFECT* WITH THE BARRED PLYMOUTH ROCK (B.P.R.) AND SINGLE COMB WHITE LEGHORN (S.C.W.L.) SEMEN DURING WEEK-1 OF HATCHABILITY

Breed of Semen Inseminated	Mean Percent Hatchability
S.C.W.L.	54.85
B.P.R.	68.96

*Probability less than .05

a, b - Statistical significance utilizing Tukey's Test (probability less than .05) is denoted by different letters in rows or columns.

Table 23

TREND DURING WEEK-3 OF HATCHABILITY WITH BARRED PLYMOUTH ROCK (B.P.R.) SEMEN INDICATING INCREASED HATCHABILITY WITH INCREASED NUMBER OF SPERMATOZOA INSEMINATED

Number of Spermatozoa Inseminated (millions)	Mean Percent Hatchability
25-1:1[1]	11.25
50-1:1	39.38
100-1:1	59.34
100-Neat	56.60

Table 24

INTERACTION** ON DURATION OF FERTILITY BETWEEN SINGLE COMB WHITE LEGHORN (S.C.W.L.) OR BARRED PLYMOUTH ROCK (B.P.R.) SEMEN AND NUMBER OF SPERMATOZOA INSEMINATED

Number of Spermatozoa Inseminated (millions)	Duration of Fertility (Days) S.C.W.L.	B.P.R.
12.5	17.75def	10.92a
25	15.08bcde	12.08ab
50[2]	20.91f	14.16abcd
100	13.25abc	17.41def

**Probability less than .01

[1]Semen was diluted 1:1 with Beltsville Extender.

[2]Peak duration of fertility is reached with 50 million S.C.W.L. spermatozoa.

a-f - Statistical significance utilizing Tukey's Test (probability less than .01) is denoted by different letters in rows or columns.

Table 25

ROUTE EFFECT* WITH THE INTRAMAGNAL (I.M.) AND INTRAVAGINAL (I.V.) ROUTE OF INSEMINATION

Route of Insemination	Duration of Fertility (Days)
I.V.	13.28
I.M.	16.16

Table 26

TREND DURING WEEK-3 OF FERTILITY WITH BARRED PLYMOUTH ROCK SEMEN INDICATING INCREASED FERTILITY WITH INCREASED NUMBERS OF SPERMATOZOA INSEMINATED

Number of Spermatozoa Inseminated (millions)	Mean Percent Fertility
25-1:1[1]	13.44
50-1:1	18.91
100-1:1	38.90
100-Neat	51.17

* Probability less than .05

[1]Semen was diluted 1:1 with Beltsville Extender.

Table 27

INTERACTION** DURING WEEK-1 OF FERTILITY BETWEEN INTRAVAGINAL (I.V.) OR INTRAMAGNAL (I.M.) ROUTES OF INSEMINATION AND NUMBER OF SPERMATOZOA INSEMINATED

Number of Spermatozoa Inseminated (millions)	Mean Percent Fertility I.V.	I.M.
12.5	27.73a	67.91cde
25	52.54bc	60.54bcde
50	75.90e	55.18bcd
100	64.50cde	42.37ab

Table 28

ROUTE EFFECT* DURING WEEK-2 OF FERTILITY WITH INTRAMAGNAL (I.M.) AND INTRAVAGINAL (I.V.) ROUTES OF INSEMINATION FOR BOTH THE SINGLE COMB WHITE LEGHORN (S.C.W.L.) AND BARRED PLYMOUTH ROCK (B.P.R.) SEMEN

Route of Insemination	Mean Percent Fertility S.C.W.L.	B.P.R.
I.V.	31.82a	26.99a
I.M.	69.00b	31.36a

Table 29

BREED EFFECT** DURING WEEK-3 OF FERTILITY AND HATCHABILITY WITH SINGLE COMB WHITE LEGHORN (S.C.W.L.) AND BARRED PLYMOUTH ROCK (B.P.R.) SEMEN

Breed of Semen Inseminated	Mean Percent Fertility	Hatchability
S.C.W.L.	52.52	45.37
B.P.R.	9.22	20.48

*Probability less than .05
**Probability less than .01
a-f - Statistical significance utilizing Tukey's Test (probability less than .01 or .05, respectively) is denoted by different letters in rows or columns.

Table 30

INTERACTION** DURING WEEK-2 OF HATCHABILITY BETWEEN ROUTE OF INSEMINATION (INTRAMAGNAL - I.M. OR INTRAVAGINAL - I.V.) AND NUMBER OF SPERMATOZOA INSEMINATED

Number of Spermatozoa Inseminated (millions)	Mean Percent Hatchability I.V.	I.M.
12.5	11.25a	58.03bc
25	53.66b	52.76b
50	87.22d	49.49b
100	82.32cd	40.61b

Table 31

INTERACTION* ON DURATION OF FERTILITY BETWEEN SINGLE COMB WHITE LEGHORN (S.C.W.L.) OR BARRED PLYMOUTH ROCK (B.P.R.) SEMEN AND ROUTE OF INSEMINATION (INTRAMAGNAL - I.M. OR INTRAVAGINAL - I.V.)

Route of Insemination	Duration of Fertility (Days) S.C.W.L.	B.P.R.
I.V.	11.06a	10.75a
I.M.	20.18b	11.25a

*Probability less than .05
**Probability less than .01

a-d - Statistical significance utilizing Tukey's Test (probability less than .01 or .05, respectively) is denoted by different letters in rows or columns.

Table 32

NUMBER EFFECT* ON DURATION OF FERTILITY WITH INCREASED NUMBERS OF SPERMATOZOA INSEMINATED

Number of Spermatozoa Inseminated (millions)	Duration of Fertility (Days)
12.5	11.41
25	13.45
50	14.88
100	16.71

Table 33

EXPERIMENTAL DESIGN OF TRIALS IIA, IIB[1]

Dilutions of Semen for Intravaginal Inseminations			Dilutions of Semen for Intramagnal Inseminations		
Neat	1:1	1:4	Neat	1:1	1:4
Number of Spermatozoa Inseminated (millions)			Number of Spermatozoa Inseminated (millions)		
12.5	12.5	12.5	12.5	12.5	12.5
25	25	25	25	25	25
50	50	50	50	50	50
100	100	100	100	100	100

*Probability less than .05

[1]Five female Single Comb White Leghorn hens per group were inseminated intravaginally (I.V.) or intramagnally (I.M.) with 12.5 million, 25 million, 50 million, or 100 million viable spermatozoa. The inseminations were made with pooled semen samples as pure semen (neat) or diluted 1:1 or 1:4 with Beltsville Extender.

Table 34A

ANALYSIS OF VARIANCE OF TRIAL IIA

Source	D.F.	Mean Square				
		Fertility Week -1	Hatchability Week -1	Fertility Week -2	Hatchability Week -2	Duration of Fertility
Route (R)	1	1,001.14	554.26	43,885.01**	237.35	1,567.15**
Number (N)	3	377.97	481.19	1,789.41*	174.68	93.19**
R x N	3	106.07	3,092.31*	222.73	1,090.79	5.62
Dilution (D)	2	745.29	842.09	1,426.03	3,827.70*	80.53*
R x D	2	2,572.96*	37.35	816.19	2,197.94	26.51
N x D	6	1,014.68	490.56	373.04	547.39	8.40
R x N x D	6	1,000.45	377.55	386.53	1,276.34	14.28
Residual	84	666.77	831.73	574.15	1,146.30	17.02
Corrected Total	107	721.39	822.04	1,001.79	1,152.49	34.05

* Probability less than .05
** Probability less than .01

Table 34B

ANALYSIS OF VARIANCE OF TRIAL IIB

Source	D.F.	Mean Square				
		Fertility Week -1	Hatchability Week -1	Fertility Week -2	Hatchability Week -2	Duration of Fertility
Route (R)	1	173.20	11,643.91**	18,467.25**	3,341.19*	1,106.28**
Number (N)	3	138.93	869.49	2,011.86*	2,120.32	71.43*
R x N	3	2,095.07*	381.95	1,281.69	370.41	3.66
Dilution (D)	2	160.92	146.62	404.94	1,774.36	9.61
R x D	2	188.35	53.19	2,297.78*	846.39	31.71
N x D	6	364.50	669.52	712.47	558.18	19.34
R x N x D	6	759.49	1,272.38	392.68	559.94	19.59
Residual	88	619.69	925.29	698.16	1,134.62	24.89
Corrected Total	111	620.27	980.80	917.31	1,104.59	34.58

* Probability less than .05
** Probability less than .01

Table 35

INTERACTION* DURING WEEK-1 OF FERTILITY BETWEEN ROUTE OF INSEMINATION (INTRAMAGNAL - I.M. OR INTRAVAGINAL - I.V.) AND DILUTION OF SEMEN (PURE SEMEN - NEAT, OR DILUTED 1:1 OR 1:4 WITH BELTSVILLE EXTENDER).

Dilution of Semen Inseminated	Mean Percent Fertility	
	I.V.	I.M.
Neat	57.52a	70.72ab
1:1	75.93ab	66.31ab
1:4	83.65b	62.67ab

Table 36

ROUTE EFFECT** DURING WEEK-2 OF FERTILITY WITH INTRAMAGNAL (I.M.) AND INTRAVAGINAL (I.V.) ROUTES OF INSEMINATION

Route of Insemination	Mean Percent Fertility
I.V.	33.40
I.M.	73.72

Table 37

NUMBER EFFECT* DURING WEEK-2 OF FERTILITY WITH INCREASED NUMBERS OF SPERMATOZOA INSEMINATED

Numbers of Spermatozoa Inseminated (millions)	Mean Percent Fertility
12.5	42.80
25	53.08
50	56.74
100	62.06

*Probability less than .05
**Probability less than .01

a,b - Statistical significance utilizing Tukey's Test (probability less than .05) is denoted by different letters in rows or columns.

Table 38

NUMBER EFFECT* DURING WEEK-3 OF FERTILITY WITH INCREASED NUMBERS OF SPERMATOZOA INSEMINATED

Number of Spermatozoa Inseminated (millions)	Mean Percent Fertility
12.5	31.74
25	36.16
50	54.90
100	59.51

Table 39

INTERACTION* DURING WEEK-1 OF HATCHABILITY BETWEEN ROUTE OF INSEMINATION (INTRAMAGNAL - I.M. OR INTRAVAGINAL - I.V.) AND NUMBER OF SPERMATOZOA INSEMINATED

Number of Spermatozoa Insemination (millions)	Mean Percent Hatchability I.V.	I.M.
12.5	47.19a	65.81ab
25	58.75a	61.31ab
50	67.40ab	62.64ab
75	81.82b	49.95a

Table 40

DILUTION EFFECT* ON HATCHABILITY DURING WEEK-2 WITH SEMEN DILUTED 1:1 AND 1:4 WITH BELTSVILLE EXTENDER

Dilution of Spermatozoa Inseminated	Mean Percent Hatchability
Neat	46.53
1:1	65.46
1:4	63.35

*Probability less than .05
**Probability less than .01
a,b - Statistical significance utilizing Tukey's Test (probability less than .05) is denoted by different letters in rows or columns.

Table 41

DILUTION EFFECT* ON HATCHABILITY DURING WEEK-3 WITH SEMEN DILUTED 1:1 AND 1:4 WITH BELTSVILLE EXTENDER

Dilution of Semen Inseminated	Mean Percent Hatchability
Neat	31.34
1:1	37.71
1:4	56.13

Table 42

ROUTE EFFECT* ON DURATION OF FERTILITY WITH INTRAMAGNAL INSEMINATION (I.M.) COMPARED TO INTRAVAGINAL INSEMINATION (I.V.)

Route of Semen Inseminated	Duration of Fertility (Days)
I.V.	13.45
I.M.	21.07

Table 43

NUMBER EFFECT** ON DURATION OF FERTILITY WITH INCREASED NUMBERS OF SPERMATOZOA INSEMINATED

Number of Spermatozoa Inseminated (millions	Duration of Fertility (Days)
12.5	15.11
25	16.46
50	19.33[1]
100	18.24

*Probability less than .05
**Probability less than .01

[1]Maximum duration of fertility was reached with the 50 million level of insemination.

Table 44

DILUTION EFFECT* ON DURATION OF FERTILITY AS THE SEMEN WAS DILUTED 1:1 AND 1:4 WITH BELTSVILLE EXTENDER

Dilution of Semen Inseminated	Duration of Fertility (Days)
Neat	15.57
1:1	18.24
1:4	18.11

Table 45

INTERACTION* DURING WEEK-1 OF FERTILITY BETWEEN ROUTE OF INSEMINATION (INTRAMAGNAL - I.M. OR INTRAVAGINAL - I.V.) AND NUMBER OF SPERMATOZOA INSEMINATED

Number of Spermatozoa Inseminated (millions)	Mean Percent Fertility I.V.	I.M.
12.5	64.40a	87.48b
25	76.10ab	72.24ab
50	79.51ab	65.34a
100	77.94ab	64.51a

Table 46

ROUTE EFFECT** DURING WEEK-2 OF FERTILITY WITH INTRAMAGNAL (I.M.) COMPARED TO THE INTRAVAGINAL (I.V.) INSEMINATIONS

Route of Semen Inseminated	Mean Percent Fertility
I.V.	39.57
I.M.	65.25

*Probability less than .05
**Probability less than .01

a, b - Statistical significance utilizing Tukey's Test (probability less than .05) is denoted by different letters in rows or columns.

Table 47

NUMBER EFFECT* DURING WEEK-2 OF FERTILITY ON FERTILITY WITH INCREASED NUMBERS OF SPERMATOZOA INSEMINATED

Number of Spermatozoa Inseminated (millions)	Mean Percent Fertility
12.5	51.97
25	44.21
50	49.19
100	64.21

Route 48

INTERACTION* DURING WEEK-2 OF FERTILITY BETWEEN ROUTE OF INSEMINATION (INTRAMAGNAL - I.M. OR INTRAVAGINAL - I.V.) AND DILUTION OF SEMEN INSEMINATED AS PURE SEMEN (NEAT) OR DILUTED 1:1 OR 1:4 WITH BELTSVILLE EXTENDER

Dilution of Semen Inseminated	Mean Percent Fertility I.V.	I.M.
Neat	35.78a	75.69c
1:1	37.55a	66.87bc
1:4	44.81ab	53.74abc

Table 49

ROUTE EFFECT** DURING WEEK-1 OF HATCHABILITY WITH INTRAVAGINAL (I.V.) AND INTRAMAGNAL (I.M.) ROUTES OF INSEMINATION

Route of Semen Inseminated	Mean Percent Hatchability
I.V.	71.84
I.M.	51.45

*Probability less than .05
**Probability less than .01

a-c - Statistical significance utilizing Tukey's Test (probability less than .05) is denoted by different letters in rows or columns.

Table 50

ROUTE EFFECT* DURING WEEK-2 OF HATCHABILITY WITH INTRAMAGNAL (I.M.) AND INTRAVAGINAL (I.V.) ROUTES OF INSEMINATION

Route of Semen Inseminated	Mean Percent Hatchability
I.V.	48.03
I.M.	58.96

Table 51

ROUTE EFFECT** ON DURATION OF FERTILITY WITH INTRAMAGNAL (I.M.) AND INTRAVAGINAL (I.V.) INSEMINATIONS

Route of Semen Inseminated	Duration of Fertility (Days)
I.V.	12.96
I.M.	19.25

Table 52

NUMBER EFFECT* ON DURATION OF FERTILITY WITH INCREASED NUMBERS OF SPERMATOZOA INSEMINATED

Number of Spermatozoa per Insemination (millions)	Duration of Fertility (Days)
12.5	15.26
25	14.78
50	15.93
100	18.39

*Probability less than .05
**Probability less than .01

Table 53

EXPERIMENTAL DESIGN OF TRIALS IIIA & IIIB[1]

Intervals of Insemination[2] with 25 million Viable Spermatozoa per Insemination				Intervals of Insemination[2] with 75 million Viable Spermatozoa per Insemination			
Time 0	Time 1 hour later	Time 0	Time 24 hrs. later	Time 0	Time 1 hr. later	Time 0	Time 24 hrs. later
S.C.W.L.	B.P.R	S.C.W.L.	B.P.R	S.C.W.L.	B.P.R.	S.C.W.L.	B.P.R.
S.C.W.L.	N.H.	S.C.W.L.	N.H.	S.C.W.L.	N.H.	S.C.W.L.	N.H.
B.P.R.	S.C.W.L.	B.P.R.	S.C.W.L.	B.P.R.	S.C.W.L.	B.P.R.	S.C.W.L.
B.P.R.	N.H.	B.P.R.	N.H.	B.P.R.	N.H.	B.P.R.	N.H.
N.H.	S.C.W.L.	N.H.	S.C.W.L.	N.H.	S.C.W.L.	N.H.	S.C.W.L.
N.H.	B.P.R.	N.H.	B.P.R.	N.H.	B.P.R.	N.H.	B.P.R.
First Breed	Second Breed	First Breed	Second Breed	First Breed	Second Breed	First Breed	Second Breed
BREED SEQUENCE OF INSEMINATION							

[1]Four New Hampshire females per group were intravaginally inseminated with 25 million or 75 million viable spermatozoa. Semen was collected from Sincle Comb White Leghorn (S.C.W.L.) males, Barred Plymouth Rock males (B.P.R.) or New Hampshire males (N.H.).

[2]Inseminations were made at various intervals with the first breed being inseminated at time 0 and the second insemination being made either 1 hour later or 24 hours later.

Table 54A

ANALYSIS OF VARIANCE OF TRIAL IIIA

Source	D.F.	Mean Square				
		Fertility Week-1	Hatchability Week-1	Fertility Week-2	Hatchability Week-2	Duration of Fertility
Breed (B)	5	893.35	1,172.16	1,448.37	1,628.06	13.34
Number (N)	1	2,230.11*	1,880.89	8,257.16**	4,797.16**	280.16*
B x N	5	798.08	727.53	1,046.88	684.13	33.51
Interval (I)	1	14.69	201.92	10.92	169.81	2.04
B x I	5	415.21	677.24	924.74	1,127.11	21.84
N x I	1	203.17	713.67	59.11	332.66	4.16
B x N x I	5	144.81	503.20	235.26	1,129.00	13.81
Residual	72	556.10	643.94	562.78	1,133.46	19.44
Corrected Total	95	565.73	679.59	706.56	1,155.17	22.09

* Probability less than .05

** Probability less than .01

Table 54B

ANALYSIS OF VARIANCE OF TRIAL IIIB

Source	D.F.	Mean Square				
		Fertility Week-1	Hatchability Week-1	Fertility Week-2	Hatchability Week-2	Duration of Fertility
Breed (B)	5	1,484.75	552.05	1,511.28	4,285.09**	99.18**
Number (N)	1	5,999.10*	1,405.68	6,040.75**	5,817.26*	237.51**
B x N	5	727.05	469.66	844.66	2,578.38	8.58
Interval (I)	1	73.69	643.61	56.15	1,018.55	19.26
B x I	5	1,966.57	1,526.66	1,535.24	1,478.67	86.48*
N x I	1	3,843.95	982.33	156.67	1,924.42	3.01
B x N x I	5	319.80	1,157.22	229.46	855.41	6.53
Residual	72	1,012.34	1,042.63	731.84	1,216.61	25.92
Corrected Total	95	1,108.38	1,017.15	837.36	1,498.35	32.94

* Probability less than .05

** Probability less than .01

Table 55

EXPERIMENTAL DESIGN OF TRIALS IIIC & IIID[1]

Intervals of Insemination with 25 million Viable Spermatozoa per Insemination			Intervals of Insemination with 75 million Viable Spermatozoa per Insemination		
Time 0	Time 24 hrs. later	Time 48 hrs. later	Time 0	Time 24 hrs. later	Time 48 hrs. later
N.H.	B.P.R.	S.C.W.L.	N.H.	B.P.R.	S.C.W.L.
N.H.	S.C.W.L.	B.P.R.	N.H.	S.C.W.L.	B.P.R.
S.C.W.L.	B.P.R.	N.H.	S.C.W.L.	B.P.R.	N.H.
S.C.W.L.	N.H.	B.P.R.	S.C.W.L.	N.H.	B.P.R.
B.P.R.	S.C.W.L.	N.H.	B.P.R.	S.C.W.L.	N.H.
B.P.R.	N.H.	S.C.W.L.	B.P.R.	N.H.	S.C.W.L.
First Breed	Second Breed	Third Breed	First Breed	Second Breed	Third Breed
BREED SEQUENCE OF INSEMINATION					

Table 56

NUMBER EFFECT* DURING WEEK-1 OF FERTILITY WITH THE 25 MILLION AND 75 MILLION LEVELS OF INSEMINATION

Number of Spermatozoa per Insemination (millions)	Mean Percent Fertility
25	68.10
75	77.74

*Probability less than .05

[1]Four New Hampshire females per group were intravaginally inseminated with 25 million or 75 million viable spermatozoa with semen from Single Comb White Leghown (S.C.W.L.), Barred Plymouth Rock (B.P.R.) or New Hampshire males (N.H.) in sequence at 24 hr. intervals starting time-0.

Table 57

NUMBER EFFECT** DURING WEEK-2 OF FERTILITY WITH THE 25 MILLION AND 75 MILLION LEVELS OF INSEMINATION

Number of Spermatozoa per Insemination (millions)	Mean Percent Fertility
25	42.98
75	61.53

Table 58

NUMBER EFFECT* DURING WEEK-2 OF HATCHABILITY WITH THE 25 MILLION AND 75 MILLION LEVELS OF INSEMINATION

Number of Spermatozoa per Insemination (millions)	Mean Percent Hatchability
25	55.58
75	69.73

Table 59

NUMBER EFFECT** ON DURATION OF FERTILITY WITH 75 MILLION VIABLE SPERMATOZOA COMPARED TO THE 25 MILLION LEVEL OF INSEMINATION

Number of Spermatozoa per Insemination (millions)	Duration of Fertility (Days)
25	12.90
75	16.31

*Probability less than .05
**Probability less than .01

Table 60

RATIOS OF CHICKS HATCHED DURING WEEK-1 FROM THE FIRST BREED INSEMINATED SHOWING THE INTERACTIONS* BETWEEN SINGLE COMB WHITE LEGHORN (S.C.W.L.), BARRED PLYMOUTH ROCK (B.P.R.) OR NEW HAMPSHIRE (N.H.) SEMEN AND NUMBER OF VIABLE SPERMATOZOA AT VARIOUS INTERVALS OF INSEMINATION.[3]

Number of Spermatozoa per Insemination		25 million		75 million	
Intervals of Insemination		0-1 hr.	0-24 hrs.	0-1 hr.	0-24 hrs.
Sequence of Insemination 1st breed	2nd Breed				
S.C.W.L.	B.P.R.	.408	.412	.150	.300
S.C.W.L.	N.H.[1]	.708	.575	.746	.111
B.P.R.	S.C.W.L.[2]	.200	.233	.300	.286
B.P.R.	N.H.	.917	.350	.775	.263
N.H.	S.C.W.L.[2]	1.000	.100	.274	.283
N.H.	B.P.R.	.475	.700	.350	.417

*Probability less than .05

[1]High ratios of first breed chicks hatched occurred when N.H. semen was used as the second breed inseminated.

[2]Note how the B.P.R. and N.H. ratios are low when S.C.W.L. semen is used as the second breed inseminated.

[3]Interactions, although existent, are not easily illustrated. Therefore, only certain trends (footnotes 1, 2) are presented.

Table 61

RATIOS OF CHICKS HATCHED DURING WEEK-2 FROM THE FIRST BREED INSEMINATED SHOWING THE INTERACTIONS* BETWEEN SEMEN OF THE SINGLE COMB WHITE LEGHORN (S.C.W.L.), BARRED PLYMOUTH ROCK (B.P.R.) OR NEW HAMPSHIRE (N.H.) AND THE NUMBER OF VIABLE SPERMATOZOA AT VARIOUS INTERVALS OF INSEMINATION[3]

Number of Spermatozoa per Insemination		25 million		75 million	
Intervals of Insemination		0-1 hr.	0-24 hrs.	0-1 hr.	0-24 hrs.
Sequence of Insemination 1st Breed	2nd Breed				
S.C.W.L.	B.P.R.	.278	.246	.263	.161
S.C.W.L.	N.H.	.833[1]	.500	.667	.555
B.P.R.	S.C.W.L.	.500	.333	.425	.567
B.P.R.	N.H.	.125	.750	.875[2]	.000
N.H.	S.C.W.L.	.550	.450	.412	.154
N.H.	B.P.R.	.458	.500	.188	.292

*Probability less than .05

[1,2]Note the high ratios of S.C.W.L. or B.P.R. chicks hatched when the N.H. semen was utilized as the second breed in the 0-1 hr. interval of insemination.

[3]Interactions, although existent, are not easily illustrated. Therefore, only certain trends (footnotes 1, 2) are presented.

Table 62

BREED EFFECT* ON RATIOS OF FIRST BREED CHICKS HATCHED FOR THE TOTAL TRIAL WHEN SINGLE COMB WHITE LEGHORN (S.C.W.L.), BARRED PLYMOUTH ROCK (B.P.R.) OR NEW HAMPSHIRE (N.H.) SEMEN WAS USED FOR THE SECOND BREED INSEMINATED

Sequence of Insemination 1st Breed	2nd Breed	Ratios of First Breed Chicks
S.C.W.L.	B.P.R.	.318
S.C.W.L.	N.H.	.628
B.P.R.	S.C.W.L.	.360
B.P.R.	N.H.	.533
N.H.	S.C.W.L.	.408
N.H.	B.P.R.	.399

Table 63

NUMBER EFFECT* ON RATIOS OF FIRST BREED CHICKS HATCHED WITH 25 MILLION OR 75 MILLION VIABLE SPERMATOZOA INSEMINATED

Number of Spermatozoa per Insemination (millions)	Ratios of First Breed Chicks
25	.509
75	.377

*Probability less than .05

Table 64

INTERVAL EFFECT** ON RATIOS OF FIRST BREED CHICKS HATCHED WHEN INSEMINATED AT THE 0-1 HOUR INTERVAL COMPARED TO THE 0-24 HOUR INTERVAL

Intervals of Insemination	Ratios of First Breed Chicks
0- 1 hr.	.522
0-24 hrs.	.358

Table 65

RATIOS OF CHICKS HATCHED DURING WEEK-1 FROM THE SECOND BREED INSEMINATED SHOWING THE INTERACTIONS* BETWEEN SEMEN OF SINGLE COMB WHITE LEGHORN (S.C.W.L.), BARRED PLYMOUTH ROCK (B.P.R.) OR NEW HAMPSHIRE (N.H.) AND NUMBER OF VIABLE SPERMATOZOA AT VARIOUS INTERVALS OF INSEMINATION[4]

Number of Spermatozoa per Insemination		25 million		75 million[2]	
Intervals of Insemination		0-1 hr.	0-24 hrs.[3]	0-1 hr.	0-24 hrs.
Sequence of Insemination 1st breed	2nd breed				
S.C.W.L.	B.P.R.	.592	.588	.850	.700
S.C.W.L.	N.H.	.292	.425	.254	.889
B.P.R.	S.C.W.L.[1]	.800	.767	.700	.714
B.P.R.	N.H.	.083	.650	.225	.738
N.H.	S.C.W.L.[1]	.000	.900	.726	.717
N.H.	B.P.R.	.525	.300	.650	.583

*Probability less than .05
**Probability less than .01

[1]Breed effect with greater numbers of second breed chicks being hatched when S.C.W.L. semen was used as the second breed inseminated.

[2]Number effect showing the 75 million level of insemination having higher ratios of chicks hatched.

[3]Interval effect showing higher ratios when inseminated at the 0-24 hours interval.

[4]Interactions, although existent, are not easily illustrated. Therefore, only certain trends (footnotes 1, 2, 3) are presented.

Table 66

RATIOS OF CHICKS HATCHED DURING WEEK-2 FROM THE SECOND BREED INSEMINATED SHOWING THE INTERACTION* BETWEEN SEMEN OF SINGLE COMB WHITE LEGHORN (S.C.W.L.), BARRED PLYMOUTH ROCK (B.P.R.) OR NEW HAMPSHIRE (N.H.) AND NUMBER OF VIABLE SPERMATOZOA AT VARIOUS INTERVALS OF INSEMINATION [3]

Number of Spermatozoa per Insemination		25 million		75 million	
Intervals of Insemination		0-1 hr.	0-24 hrs.	0-1 hr.	0-24 hrs.
Sequence of Insemination 1st breed	2nd breed				
S.C.W.L.	B.P.R.	.722	.754	.737	.839
S.C.W.L.	N.H.	.167[1]	.500	.333[1]	.444
B.P.R.	S.C.W.L.	.500	.667	.575	.433
B.P.R.	N.H.	.875	.250[2]	.062[2]	1.000
N.H.	S.C.W.L.	.450	.550	.588	.846
N.H.	B.P.R.	.542	.500	.812	.708

*Probability less than .05

[1],[2]Breed effect showing lower ratios when N.H. semen was used as the second breed inseminated.

[3]Interactions, although existent, are not easily illustrated. Therefore, only certain trends (footnotes 1, 2) are presented.

Table 67

RATIOS OF CHICKS HATCHED FOR THE TOTAL TRIAL FROM THE SECOND BREED INSEMINATED SHOWING THE INTERACTION* BETWEEN SEMEN OF THE SINGLE COMB WHITE LEGHORN (S.C.W.L.), BARRED PLYMOUTH ROCK (B.P.R.) OR NEW HAMPSHIRE (N.H.) AND VARIOUS INTERVALS OF INSEMINATION[3]

Sequence of Inseminations		Intervals of Insemination	
1st breed	2nd breed	0-1 hr.	0-24 hrs.[2]
S.C.W.L.	B.P.R.	.650	.714
S.C.W.L.	N.H.[1]	.288	.456
B.P.R.	S.C.W.L.	.653	.628
B.P.R.	N.H.	.240	.680
N.H.	S.C.W.L.	.418	.765
N.H.	B.P.R.	.626	.567

Table 68

NUMBER EFFECT* FOR TOTAL TRIAL ON RATIOS OF SECOND BREED CHICKS HATCHED WITH 25 MILLION AND 75 MILLION VIABLE SPERMATOZOA PER INSEMINATION

Number of Spermatozoa per Insemination (millions)	Ratios of Second Breed Chicks
25	.491
75	.621

*Probability less than .05

[1]Breed effect - the ratios indicate lower numbers of second breed chicks were hatched when N.H. semen was used as the second breed inseminated.

[2]Interval effect - the 0-24 hour interval was superior to the 0-1 hour interval.

[3]Interactions, although existent, are not easily illustrated. Therefore, only certain trends (footnotes 1, 2) are presented.

Table 69

NUMBER EFFECT* ON DURATION OF FERTILITY WITH 25 MILLION AND 75 MILLION VIABLE SPERMATOZOA PER INSEMINATION

Number of Spermatozoa per Insemination (millions)	Duration of Fertility (Days)
25	12.9
75	16.3

Table 70

NUMBER EFFECT* DURING WEEK-1 OF FERTILITY WITH 25 MILLION AND 75 MILLION SPERMATOZOA INSEMINATED

Number of Spermatozoa per Insemination (millions)	Mean Percent Fertility
25	56.91
75	72.72

Table 71

NUMBER EFFECT* DURING WEEK-2 OF FERTILITY WITH 25 MILLION AND 75 MILLION SPERMATOZOA INSEMINATED

Number of Spermatozoa per Insemination (millions)	Mean Percent Fertility
25	29.51
75	45.38

*Probability less than .05

Table 72

BREED EFFECT** DURING WEEK-2 OF HATCHABILITY WHEN SINGLE COMB WHITE LEGHORN (S.C.W.L.), NEW HAMPSHIRE (N.H.) AND BARRED PLYMOUTH ROCK (B.P.R.) SEMEN WAS USED AS THE SECOND BREED INSEMINATED

Sequence of Insemination		Mean Percent Hatchability
1st breed	2nd breed	
S.C.W.L.	B.P.R.	61.39
S.C.W.L.	N.H.	36.56
B.P.R.	S.C.W.L.[1]	63.17
B.P.R.	N.H.	31.67
N.H.	S.C.W.L.[1]	72.51
N.H.	B.P.R.	44.67

Table 73

NUMBER EFFECT* DURING WEEK-2 OF HATCHABILITY WITH 25 MILLION AND 75 MILLION VIABLE SPERMATOZOA

Number of Spermatozoa per Insemination (millions)	Mean Percent Hatchability
25	43.85
75	59.45

*Probability less than .05
**Probability less than .01

[1]Note the higher hatchability when the S.C.W.L. semen was used as the second breed inseminated.

Table 74

INTERACTION* ON DURATION OF FERTILITY BETWEEN SINGLE COMB WHITE LEGHORN (S.C.W.L.), BARRED PLYMOUTH ROCK (B.P.R.) OR NEW HAMPSHIRE (N.H.) SEMEN AND VARIOUS INTERVALS OF INSEMINATION

Intervals of Insemination		0-1 hr.	0-24 hrs.
Sequence of Insemination 1st breed	2nd breed	Duration of Fertility (Days)	
S.C.W.L.	B.P.R.	13.5def	14 ef
S.C.W.L.	N.H.[1]	7.1ab	13.2def
B.P.R.	S.C.W.L.	9.5abcd	15.3f
B.P.R.	N.H.[1]	10.8bcde	5.4a
N.H.	S.C.W.L.	13.8ef	15.4f
N.H.	B.P.R.	11.6cdef	8.4abc

Table 75

NUMBER EFFECT** ON DURATION OF FERTILITY WITH THE 25 MILLION AND 75 MILLION LEVELS OF INSEMINATION

Number of Spermatozoa per Insemination (millions)	Duration of Fertility (Days)
25	9.9
75	13.1

*Probability less than .05
**Probability less than .01

[1]The shorter duration of fertility occurred when the N.H. was used as the second breed compared to the longer duration when the S.C.W.L. was used as the second breed inseminated.

a-f - Statistical significance utilizing Tukey's Test (probability less than .05) is denoted by different letters in rows or columns.

Table 76

RATIOS OF CHICKS HATCHED DURING WEEK-1 FROM THE FIRST BREED INSEMINATED WITH THE DOMINATING BREED EFFECT* OF SINGLE COMB WHITE LEGHORN (S.C.W.L.) SEMEN OVER BARRED PLYMOUTH ROCK (B.P.R.) AND NEW HAMPSHIRE (N.H.) SEMEN

Sequence of Insemination 1st breed	2nd breed	Ratios of First Breed Chicks
S.C.W.L.	B.P.R.	.416
S.C.W.L.	N.H.	.646
B.P.R.	S.C.W.L.	.337
B.P.R.	N.H.	.788
N.H.	S.C.W.L.	.102
N.H.	B.P.R.	.365

Table 77

RATIOS OF CHICKS HATCHED DURING WEEK-2 FROM THE FIRST BREED INSEMINATED WITH THE INTERACTIONS* BETWEEN SEMEN OF SINGLE COMB WHITE LEGHORN (S.C.W.L.), BARRED PLYMOUTH ROCK (B.P.R.) OR NEW HAMPSHIRE (N.H.) AND VARIOUS NUMBERS OF SPERMATOZOA INSEMINATED[3]

Sequence of Insemination 1st breed	2nd breed	Number of Spermatozoa per Insemination 25 million	75 million
S.C.W.L.	B.P.R.	.138	.385
S.C.W.L.	N.H.	.833	.781
B.P.R.	S.C.W.L.	.028	.643[2]
B.P.R.	N.H.	.500	.917
N.H.	S.C.W.L.	.088[1]	.060
N.H.	B.P.R.	.400	.000

*Probability less than .05

[1]Breed Effect - N.H. semen used for the first insemination had the lowest ratios, whereas, the S.C.W.L. and B.P.R. semen had higher ratios.

[2]Number Effect - The overall ratios of chicks hatched were higher at the 75 million level of insemination.

[3]Interactions, although existent, are not easily illustrated. Therefore, only certain trends (footnotes 1, 2) are presented.

Table 78

RATIOS OF CHICKS HATCHED FOR THE TOTAL TRIAL FROM THE FIRST BREED INSEMINATED WITH THE INTERACTIONS* BETWEEN SEMEN OF SINGLE COMB WHITE LEGHORN (S.C.W.L.), BARRED PLYMOUTH ROCK (B.P.R.) OR NEW HAMPSHIRE (N.H.) AND VARIOUS INTERVALS OF INSEMINATION

Sequence of Insemination		Intervals of Insemination	
1st breed	2nd breed	0-1 hr.	0-24 hrs.
S.C.W.L.	B.P.R.	.463	.304
S.C.W.L.	N.H.	.388	.847
B.P.R.	S.C.W.L.	.338	.330
B.P.R.	N.H.	.705	.938
N.H.	S.C.W.L.	.036[1]	.156[2]
N.H.	B.P.R.	.256	.432

Table 79

BREED EFFECT** ON RATIOS OF SECOND BREED CHICKS HATCHED DURING WEEK-1 WHEN THE SINGLE COMB WHITE LEGHORN (S.C.W.L.) WAS USED AS THE SECOND BREED INSEMINATED, COMPARED TO BARRED PLYMOUTH ROCK (B.P.R.) OR NEW HAMPSHIRE (N.H.) SEMEN

Sequence of Insemination		Ratios of Second Breed Chicks
1st breed	2nd breed	
S.C.W.L.	B.P.R.	.584
S.C.W.L.	N.H.	.282[4]
B.P.R.	S.C.W.L.	.663
B.P.R.	N.H.	.212[4]
N.H.	S.C.W.L.	.898
N.H.	B.P.R.	.635

*Probability less than .05
**Probability less than .01

[1],[2]Breed Effect - N.H. semen used for the first insemination had fewer chicks hatched at both the 0-1 hour interval and 0-24 hour interval of insemination.

[3]Interactions, although existent, are not easily illustrated. Therefore, only certain trends (footnotes 1, 2) are presented.

[4]Note the low ratios which occur when N.H. semen is used as the second breed inseminated.

Table 80

RATIOS OF SECOND BREED CHICKS HATCHED DURING WEEK-1 WITH THE INTERACTION* BETWEEN NUMBER OF SPERMATOZOA INSEMINATED AND INTERVAL OF INSEMINATION

Number of Spermatozoa Inseminated (millions)	Intervals of Inseminations 0-1 hr.	0-24 hrs.
25	.721b	.502a
75	.496a	.551a

Table 81

RATIOS OF SECOND BREED CHICKS HATCHED DURING WEEK-2 WITH THE INTERACTION* BETWEEN SEMEN FROM SINGLE COMB WHITE LEGHORN (S.C.W.L.), BARRED PLYMOUTH ROCK (B.P.R.) OR NEW HAMPSHIRE (N.H.) AND VARIOUS NUMBERS OF VIABLE SPERMATOZOA INSEMINATED[3]

Sequence of Insemination 1st breed	2nd breed	Number of Spermatozoa per Insemination 25 million[4]	75 million
S.C.W.L.	B.P.R.	.861[2]	.615
S.C.W.L.	N.H.	.167	.150
B.P.R.	S.C.W.L.[1]	.972	.357
B.P.R.	N.H.	.500	.083
N.H.	S.C.W.L.[1]	.912	.940
N.H.	B.P.R.	.600	.000[2]

*Probability less than .05

[1]Breed Effect - Greater numbers of chicks hatched when semen from the S.C.W.L. was used for the second insemination.

[2]Number Effect - Greater numbers of second breed chicks were hatched after inseminations with the 25 million level of spermatozoa.

[3]Interactions, although existent, are not easily illustrated. Therefore, only certain trends (footnotes 1, 2) are presented.

Table 82

BREED EFFECT** ON RATIOS OF SECOND BREED CHICKS HATCHED FOR THE TOTAL TRIAL WHEN SEMEN FROM SINGLE COMB WHITE LEGHORN (S.C.W.L.), BARRED PLYMOUTH ROCK (B.P.R.) OR NEW HAMPSHIRE (N.H.) WAS INSEMINATED AS THE SECOND BREED

Sequence of Insemination		Ratios of Second Breed
1st breed	2nd breed	Chicks
S.C.W.L.	B.P.R.	.621
S.C.W.L.	N.H.[1]	.266
B.P.R.	S.C.W.L.	.666
B.P.R.	N.H.[1]	.211
N.H.	S.C.W.L.	.904
N.H.	B.P.R.	.663

Table 83

RATIOS OF SECOND BREED CHICKS HATCHED FOR THE TOTAL TRIAL WITH THE INTERACTION* BETWEEN NUMBER OF VIABLE SPERMATOZOA INSEMINATED AND VARIOUS INTERVALS OF INSEMINATION

Number of Spermatozoa Inseminated (millions)	Interval of Insemination 0-1 hr.	0-24 hrs.
25	.749b	.524a
75	.518a	.542a

*Probability less than .05
**Probability less than .01

a, b - Statistical significance utilizing Tukey's Test (probability less than .05) is denoted by different letters in rows or columns.

[1]Note the low number of chicks hatched when the N.H. semen was used as the second breed inseminated.

Table 84

NUMBER EFFECT** ON DURATION OF FERTILITY WITH THE 25 MILLION AND 75 MILLION LEVELS OF INSEMINATION

Number of Spermatozoa per Insemination (millions)	Duration of Fertility (Days)
25	9.9
75	13.1

Table 85

INTERACTION* ON DURATION OF FERTILITY OF SEMEN FROM SINGLE COMB WHITE LEGHORN (S.C.W.L.), BARRED PLYMOUTH ROCK (B.P.R.) OR NEW HAMPSHIRE (N.H.) AND INTERVAL OF INSEMINATION

Sequence of Inseminations		Interval of Inseminations	
1st breed	2nd breed	0-1 hr.	0-24 hrs.
S.C.W.L.	B.P.R.	13.5def	14.0ef
S.C.W.L.	N.H.	7.1ab	13.2def
B.P.R.	S.C.W.L.	9.5abcd	15.2f
B.P.R.	N.H.	10.7bcde	5.4a
N.H.	S.C.W.L.	13.8ef	15.4f
N.H.	B.P.R.	11.6cdef	8.4abc

Table 86

NUMBER EFFECT* DURING WEEK-1 OF FERTILITY WITH 25 MILLION AND 75 MILLION VIABLE SPERMATOZOA INSEMINATED

Number of Spermatozoa Inseminated (millions)	Mean Percent Fertility
25	75.44
75	85.37

*Probability less than .05
**Probability less than .01
a-f - Statistical significance utilizing Tukey's Test (probability less than .05) is denoted by different letters in rows or columns.

Table 87

NUMBER EFFECT* DURING WEEK-2 OF FERTILITY WITH 25 MILLION AND 75 MILLION VIABLE SPERMATOZOA INSEMINATED

Number of Spermatozoa Inseminated (millions)	Mean Percent Fertility
25	42.98
75	58.18

Table 88

BREED EFFECT** ON DURATION OF FERTILITY IN TRIALS IIIC AND IIID WITH THE SINGLE COMB WHITE LEGHORN (S.C.W.L.), BARRED PLYMOUTH ROCK (B.P.R.) AND NEW HAMPSHIRE (N.H.) SEMEN, RESPECTIVELY

Second Breed Inseminated	Duration of Fertility (Days)	
	Trial IIIC	Trial IIID
S.C.W.L.	12.5	8.6
B.P.R.	7.2	4.5
N.H.	3.0	2.3

Table 89

NUMBER EFFECT** ON FERTILITY DURING WEEK-2 WITH 25 MILLION AND 75 MILLION VIABLE SPERMATOZOA PER INSEMINATION

Number of Spermatozoa Inseminated (millions)	Mean Percent Fertility
25	44.61
75	61.76

*Probability less than .05
**Probability less than .01

Table 90

NUMBER EFFECT* ON DURATION OF FERTILITY FOR THE FIRST BREED INSEMINATED WITH THE 25 MILLION AND 75 MILLION LEVELS OF INSEMINATION

Number of Spermatozoa per Insemination (millions)	Duration of Fertility (Days)
25	3.75
75	5.80

Table 91

BREED EFFECT** ON DURATION OF FERTILITY FOR THE FIRST BREED INSEMINATED WITH SINGLE COMB WHITE LEGHORN (S.C.W.L.), BARRED PLYMOUTH ROCK (B.P.R.) AND NEW HAMPSHIRE (N.H.) SEMEN IN BOTH TRIALS IIIC AND IIID

First Breed Inseminated	Duration of Fertility (Days)	
	Trial IIIC	Trial IIID
S.C.W.L.	8.0	3.9
B.P.R.	8.3	4.0
N.H.	2.6	1.5

Table 92

NUMBER EFFECT** ON DURATION OF FERTILITY FOR THE SECOND BREED INSEMINATED WITH 25 MILLION AND 75 MILLION VIABLE SPERMATOZOA PER INSEMINATION

Number of Spermatozoa per Insemination (millions)	Duration of Fertility (Days)
25	2.55
75	6.55

*Probability less than .05

**Probability less than .01

Table 93

NUMBER EFFECT** ON DURATION OF FERTILITY FOR THE TOTAL TRIAL WITH 25 MILLION AND 75 MILLION VIABLE SPERMATOZOA PER INSEMINATION

Number of Spermatozoa per Insemination (millions)	Duration of Fertility (Days)
25	12.92
75	15.77

Table 94

RATIOS OF CHICKS HATCHED DURING WEEK-2 FROM THE FIRST BREED INSEMINATED INDICATING A BREED EFFECT** WITH BARRED PLYMOUTH ROCK (B.P.R.), SINGLE COMB WHITE LEGHORN (S.C.W.L.) AND NEW HAMPSHIRE (N.H.) SPERMATOZOA

First Breed Inseminated	Ratios of First Breed Chicks Trial IIIC	Trial IIID
S.C.W.L.	.243	.055
B.P.R.	.369[1]	.097
N.H.	.077	.000

Table 95

RATIOS OF CHICKS HATCHED DURING WEEK-2 FROM THE THIRD BREED INSEMINATED INDICATING A BREED EFFECT** WITH BARRED PLYMOUTH ROCK (B.P.R.), SINGLE COMB WHITE LEGHORN (S.C.W.L.) AND NEW HAMPSHIRE (N.H.) SPERMATOZOA

Third Breed Inseminated	Ratios of Third Breed Chicks Trial IIIC	Trial IIID
S.C.W.L.	.603	.764
B.P.R.	.960[2]	.916
N.H.	.487	.443

*Probability less than .05
**Probability less than .01

[1]All ratios in this table are low which indicates very few chicks hatched during Week-2 were sired by the first breed inseminated.

[2]Ratios in this table are high indicating the majority of chicks hatched during Week-2 were sired by the third breed inseminated.

SUMMARY AND CONCLUSIONS

By utilizing two routes of insemination, intravaginal artificial insemination (I.V.A.I.) and intramagnal artificial insemination (I.M.A.I.), the storage potential of the uterovaginal junction (U.V.J.) host glands and infundibular crypts was studied.

The fertility data from Experiment I indicated higher fertility was achieved with I.M.A.I. compared to I.V.A.I. This was true when either Single Comb White Leghorn (S.C.W.L.) or Barred Plymouth Rock (B.P.R.) semen was inseminated (Figures 1 and 2). Fertility was greater with I.M.A.I. during weeks one, two and three when 12.5 million, 25 million or 50 million viable spermatozoa were inseminated. However, when 100 million spermatozoa were inseminated, the fertility was higher with I.M.A.I. only during weeks two and three. This may indicate that the infundibular crypts were overwhelmed by large numbers of spermatozoa during the first week of fertility when intramagnally inseminated.

The breed effect on percent fertility demonstrated that S.C.W.L. semen was superior to the B.P.R. semen at all four levels of insemination (Figure 3). The author has no complex hypothesis for this effect other than a simple breed preference or compatibility of the like-breed.

Duration of fertility was longer with I.M.A.I. when either the S.C.W.L. or B.P.R. spermatozoa was inseminated (Figures 4, 5, respectively). Data for the B.P.R. inseminations showed a linear trend (Figure 5) for both I.V.A.I. and I.M.A.I. suggesting both the uterovaginal host glands

and the infundibular crypts can accommodate and maintain all four levels of spermatozoa inseminated. On the other hand, the longer duration of fertility (Figure 4) with the S.C.W.L. at the 12.5 million and 25 million level of insemination I.M.A.I. suggested the infundibular crypts had the greater ability to maintain duration of fertility even at low numbers of inseminated semen.

Experiment II was conducted to confirm data in Experiment I and expand on the dilution effect. Intravaginal or intramagnal inseminations were made with either pure semen (neat) or semen diluted 1:1 or 1:4 with Beltsville Extender. Only S.C.W.L. males were used because of the superiority that they showed in Experiment I.

The combined data from Experiments IIA and IIB (Figure 6) confirmed the information discussed in Experiment I concerning fertility.

Of extreme importance was the dilution effect on fertility (Figure 7). During weeks one and two fertility increased as intravaginally inseminated neat semen was diluted 1:1 and 1:4 with Beltsville Extender. This may have been due to the increased dispersion of the spermatozoa throughout the U.V.J. host glands. This suggested that the increased dispersion of spermatozoa throughout the U.V.J. storage site may facilitate increased maintenance of the spermatozoa and thus increased fertilizing capabilities; whereas, neat samples could be bunched up or concentrated in one area and, therefore, were not released from the U.V.J. to fulfill their fertilizing capabilities. The opposite appeared to be true with I.M.A.I. as noted by the decrease in fertility during weeks one and two as with semen diluted 1:4 or 1:1 as compared to neat.

The route effect was significant only during the second week and showed I.M.A.I. being superior with neat and both dilutions of spermatozoa

inseminated. The route-dilution interaction pointed out the advantage of I.V.A.I. at 1:1 and 1:4 dilutions (Figure 7).

Hatchability during the first week following insemination was not affected by dilution although the dilution effect was significant during the second and third weeks of Trial IIA. Increased hatchability with the 1:1 and 1:4 dilutions was indicated for weeks two and three of Trial IIB.

Duration of fertility was longer with the diluted samples which again suggested that the increased dispersion of spermatozoa, particularly with 1:1 dilution, may be advantageous to sperm maintenance.

In Experiment III genetic markers were used in which the progeny had specific characteristic phenotypes to identify the respective sire. In essence, the sequence of chicks hatched theoretically represented the sequence of spermatozoa released from the U.V.J. host glands. Therefore, by comparing the ratios of the different breeds of chicks hatched to the total chicks hatched during the first week, second week and for the total trial, insight was gained as to the sequential releasing pattern of spermatozoa from the U.V.J. By comparing the order of spermatozoa released to the order of insemination, the role of the U.V.J. host glands as a regulator of sperm release was studied.

Several limitations of this theory are as follows: (1) Spermatozoa are released in groups. Therefore, even though one type of sperm (i.e., N.H.) fertilized the ovum, other sperm (i.e., S.C.W.L., B.P.R.) may also have been released. (2) The spermatozoa inseminated are believed to be associated with and released from the U.V.J. tubules when, in fact, an unknown number travels up to the infundibular crypts where they may be stored and released later. These limitations must be considered when

evaluation of the data is made.

A comparison of overall ratios of second breed chicks[4] hatched in Trials IIIA and IIIB indicated more spermatozoa from the second breed inseminated are released from the U.V.J. host glands during week one, week two and the overall trial. This suggested the host glands receive and store spermatozoa in an orderly fashion (i.e., N.H.-B.P.R.) and in some way facilitate the release of the same spermatozoa in a sequence opposite to their introduction (i.e., B.P.R.-N.H.).

Although the overall ratios of chicks hatched are important, they fail to point out one specialized effect which occurred within this experiment. This was a number effect during the first week of Trial IIIA in which higher numbers of first breed chicks were hatched during week one when 25 million spermatozoa were inseminated compared to 75 million spermatozoa inseminated (Table 60). Therefore, when 25 million viable spermatozoa were inseminated, the releasing sequence during the first week was opposite to the overall trend.

The addition of the third breed to the sequence of inseminations lowered the ratios of chicks hatched for each breed. However, the sequence of spermatozoa release from the U.V.J. was opposite to their introduction. Also, the introduction of a third breed to the sequence of insemination (Trials IIIC and IIID) failed to result in significant number and interval effects. The fertility and duration of fertility were superior with the 75 million level of insemination as had been the case in the two breed sequence of inseminations. Also the addition of a

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[4]Second breed chicks are those sired by semen from the second breed inseminated.

third breed to the insemination sequence, i.e., Experiment IIIC and IIID, lowered the duration of fertility for the first breed inseminated when compared to the first breed inseminated in the two breed combination. This occurred at both the 25 million and 75 million level of insemination.

APPENDICES

Appendix A

Practical Layer Ration

Nutrient	Laying Chickens
Total Protein, %	18
Vitamins	
A, I.U.	2000
D_3, I.C.U.	500
E, I.U.	10
K_1, mg	.5
Thiamine, mg.	----
Riboflaven, mg.	2
Panothenic acid, mg.	4
Niacin, mg.	12
Pyridoxine, mg.	1.5
Biotin, mg.	.05
Choline, mg.	500
Folacin, mg.	.15
Vitamine B_{12}, mg.	.005
Minerals	
Calcium, %	3.1-3.6
Phosphorus, avail. %	.5
Sodium, as salt, %	.25
Potassium, %	----
Manganese, mg.	15
Iodine, mg.	.20
Copper, mg.	2
Zinc, mg.	20
Iron, mg.	20
Selenium, mg.	----

Appendix B

Technique for Measuring Optical Density of Diluted Semen

1. Mix 5 microliters of fresh semen with 5 milliliters of physiological saline in a Fisher cuvette.

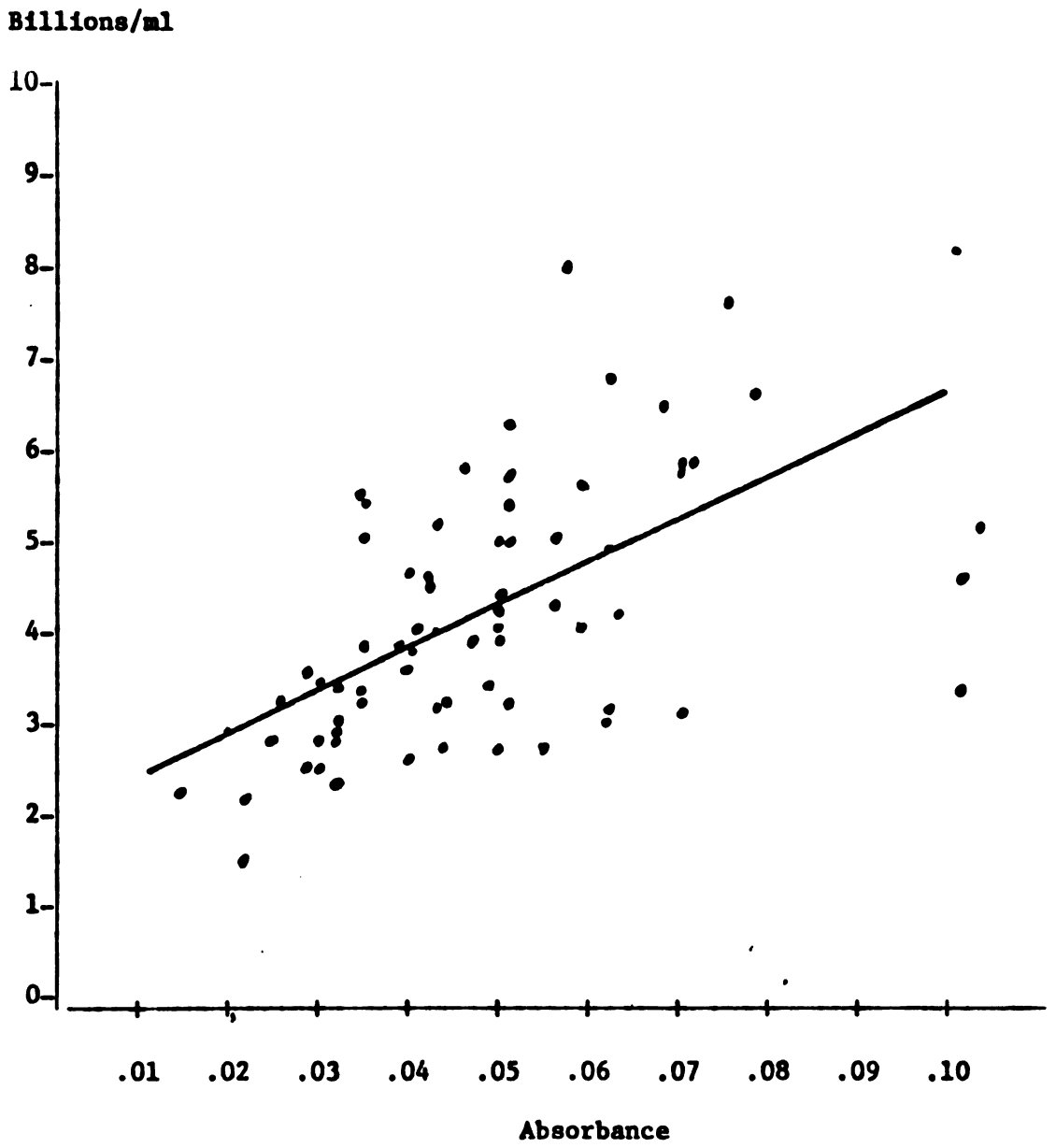
2. Place in standardized Fisher Electrophotometer (red filter- 350 millimicrons) and read optical density.

3. Count and calculate sperm numbers utilizing the standard hemacytometer technique.

4. Plot sperm numbers against the optical density and make a linear regression (Appendix C).

Appendix C

Linear Regression for Estimation of Sperm-Cell Numbers



Appendix D

Macroscopic Identification of Undeveloped Fertile Eggs

	Code	Description
1.)	03	Germinal Disc developed
2.)	04	Blood Islets formed
3.)	D.E.	Dead Embryo (1-14 days)
4.)	D.S.	Dead in shell (15-21 days)
5.)	L.S.	Live in shell
6.)	P.L.	Pipped Live
7.)	P.D.	Pipped Dead

Appendix E

Phenotypic Identification of Progeny from Parent Cross

Parent Cross		Phenotype of Offspring
Male	Female	
N.H.	N.H.	Rapid feathering Red color
S.C.W.L.	N.H.	Rapid feathering Yellow color
B.P.R.	N.H.	Slow feathering Black color

Appendix F

Parameters Analyzed in Experiment III, Trials IIIA, IIIB

1. Fertility (Week-1, days 3-9), (Week-2, days 10-16), (Total Trial)

2. Hatchability (Week-1, days 3-9), (Week-2, days 10-16), (Total Trial)

3. Duration of Fertility - Single Comb White Leghorn (S.C.W.L.), Barred Plymouth (B.P.R.) and New Hampshire (N.H.)[1]

4. Ratios of chicks hatched

   A. First breed inseminated (Week-1) (Week-2) (Total Trial)
   B. Second breed inseminated (Week-1) (Week-2) (Total Trial)

5. Variables Analyzed

   A. Breed effect (S.C.W.L. vs B.P.R. vs N.H.)
   B. Number effect (25 million/insemination vs 75 million/insemination)
   C. Interval effect (Insemination at time 0-1 hour vs 0-24 hours)
   D. Interactions

[1]Duration of fertility was measured from day of insemination until the last fertile egg was laid.

Appendix G

Parameters Analyzed in Experiment III, Trials IIIC, IIID

1. Fertility (Week-1, days 3-9) (Week-2, days 10-16) (Total Trial)

2. Hatchability (Week-1, days 3-9) (Week-2, days 10-16) (Total Trial)

3. Duration of Fertility[1] - Single Comb White Leghorn (S.C.W.L.), Barred Plymouth Rock (B.P.R.) and New Hampshire (N.H.)

4. Ratios of chicks hatched

   A. First breed inseminated (Week-1)(Week-2) (Total Trial)
   B. Second breed inseminated (Week-1) (Week-2) (Total Trial)
   C. Third breed inseminated (Week-1) (Week-2) (Total Trial)

5. Variables analyzed

   A. Breed effect (S.C.W.L. vs B.P.R. vs N.H.)
   B. Number effect (25 million/insemination vs 75 million/insemination)
   C. Interactions

[1]Duration of fertility was measured from day of insemination until the last fertile egg was laid.

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FIGURES

FIGURE 1
PERCENT FERTILITY (WEEKLY)
SCWL♂ x SCWL ♀♀

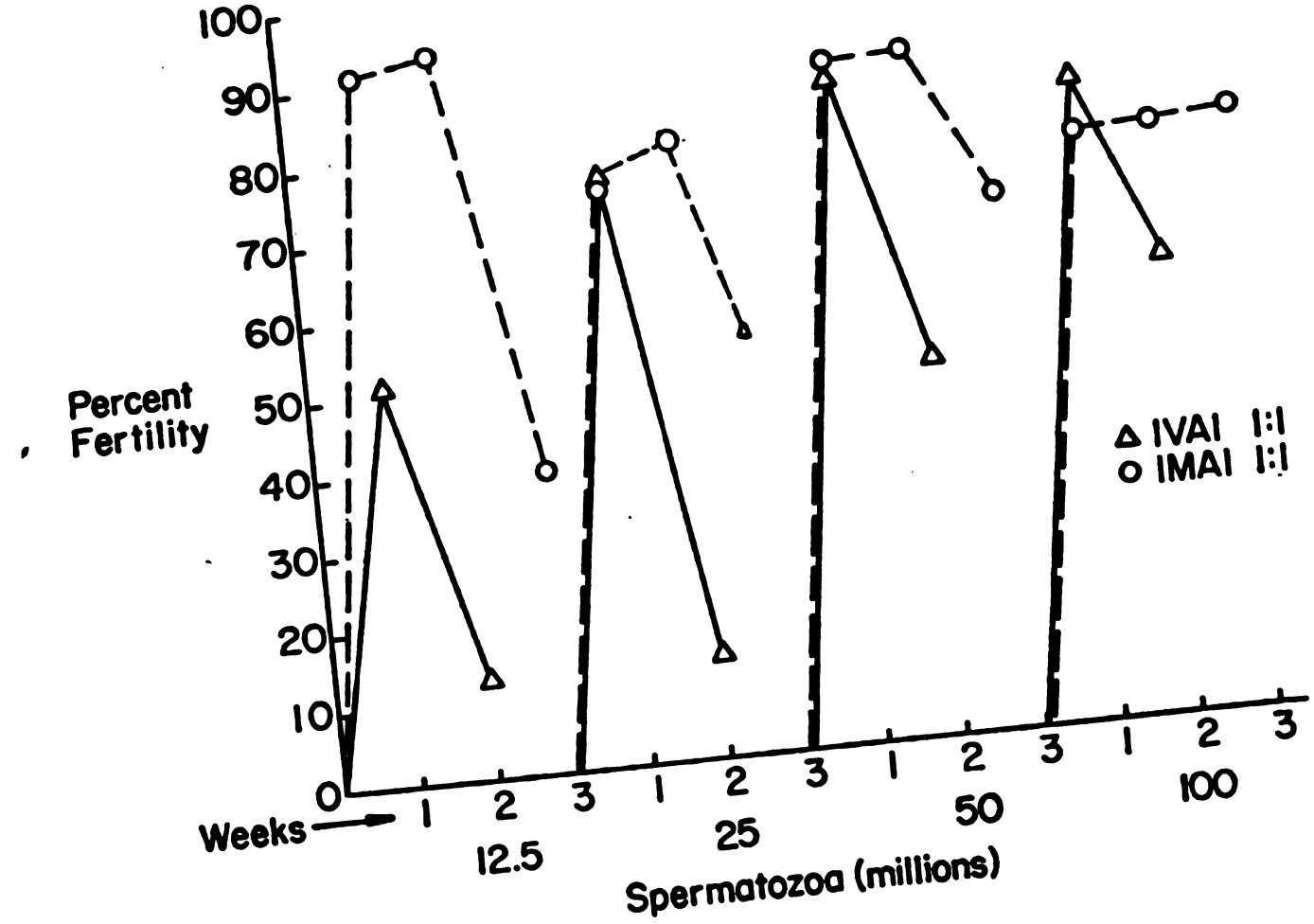


FIGURE 2
PERCENT FERTILITY (WEEKLY)
BPR ♂ x SCWL ♀♀

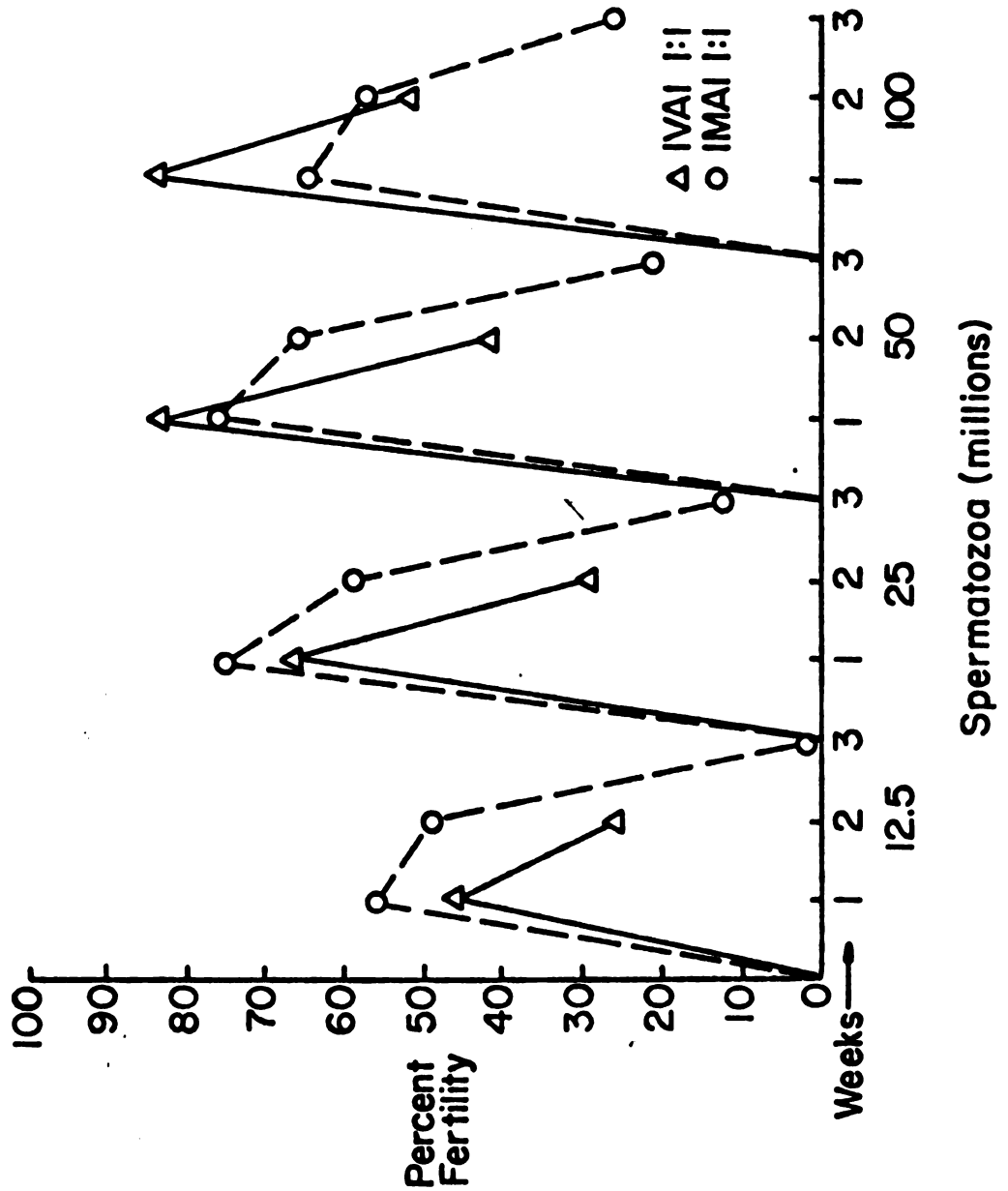
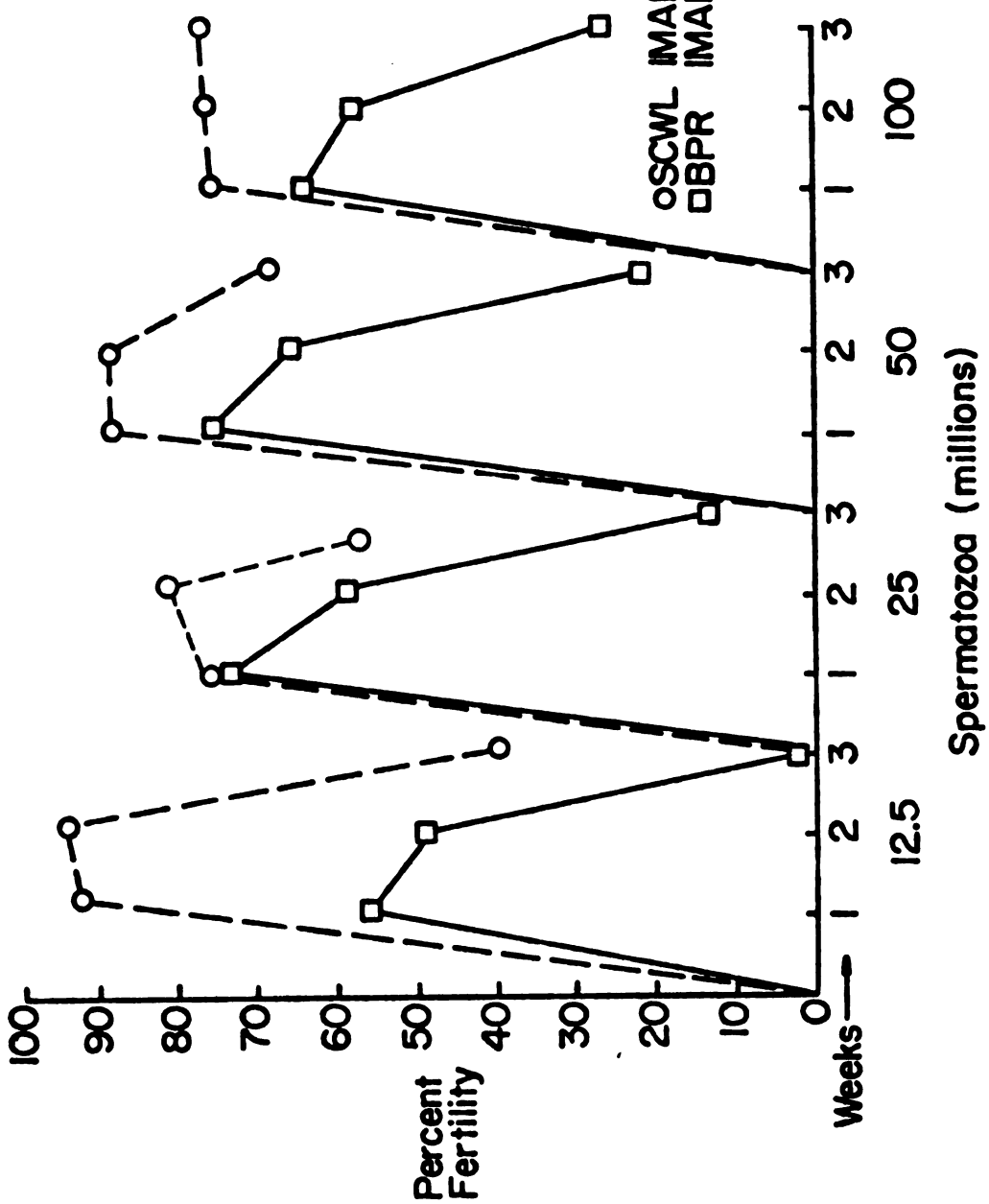


FIGURE 3
PERCENT FERTILITY (WEEKLY)
SCWL ♂ vs. BPR ♂



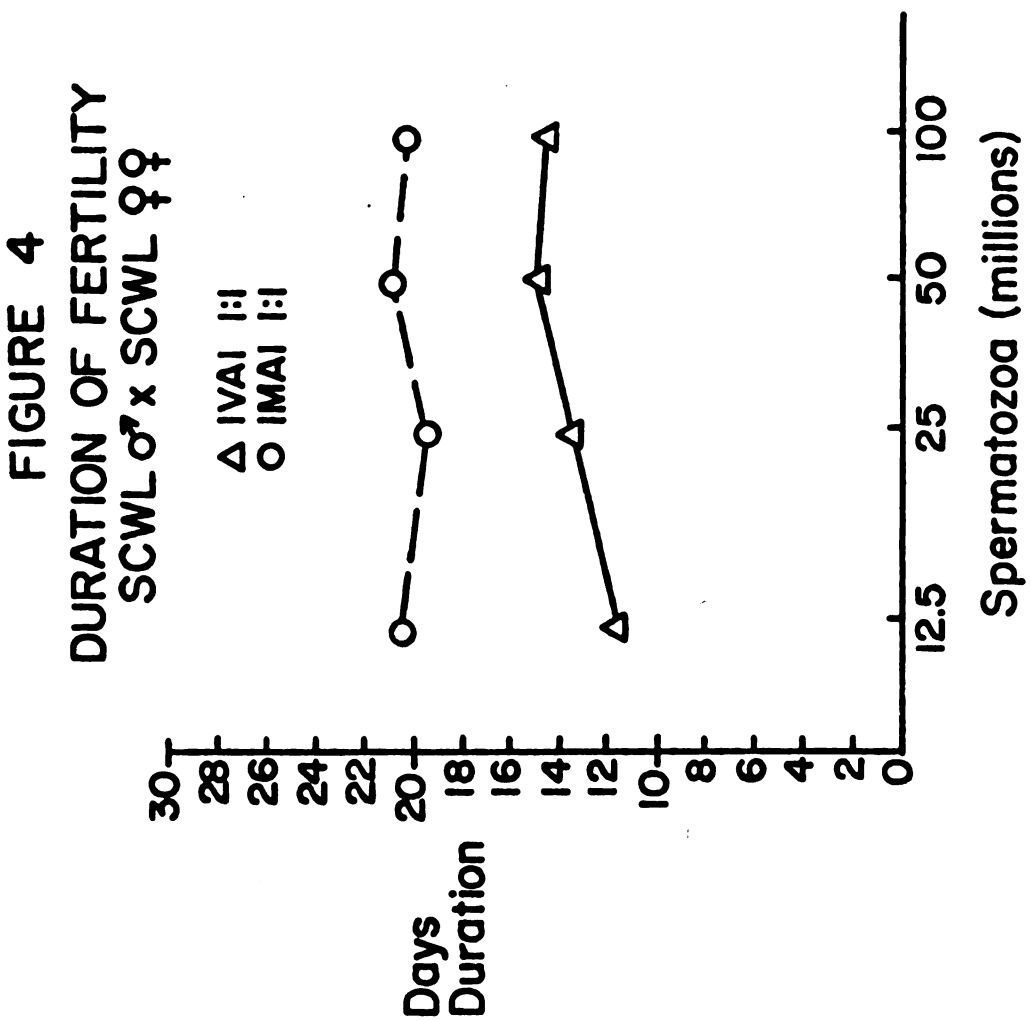


FIGURE 4
DURATION OF FERTILITY
SCWL ♂ x SCWL ♀♀
△ IVAI 1:1
○ IMAI 1:1
Days Duration
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
12.5
25
50
100
Spermatozoa (millions)

FIGURE 5

DURATION OF FERTILITY
BPR ♂ x SCWL ♀♀

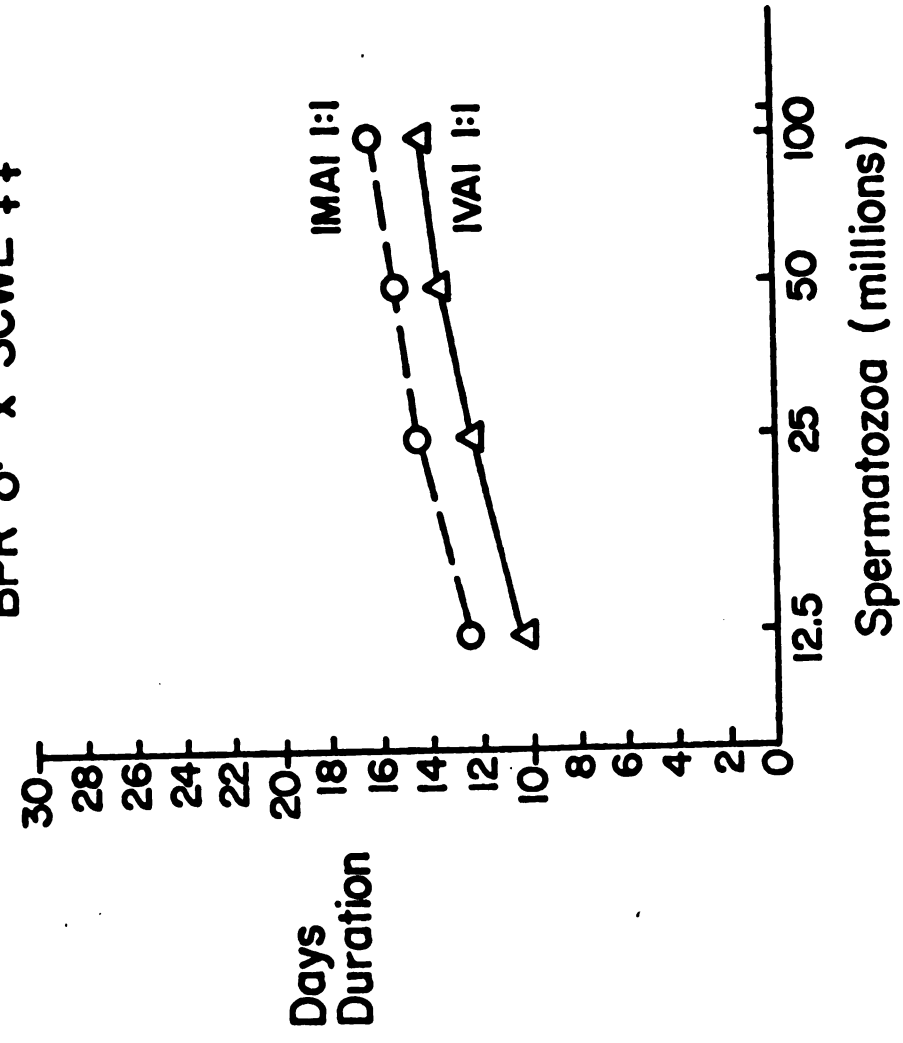


FIGURE 6
ROUTE AND NUMBER INTERACTION
PERCENT FERTILITY (WEEKLY)

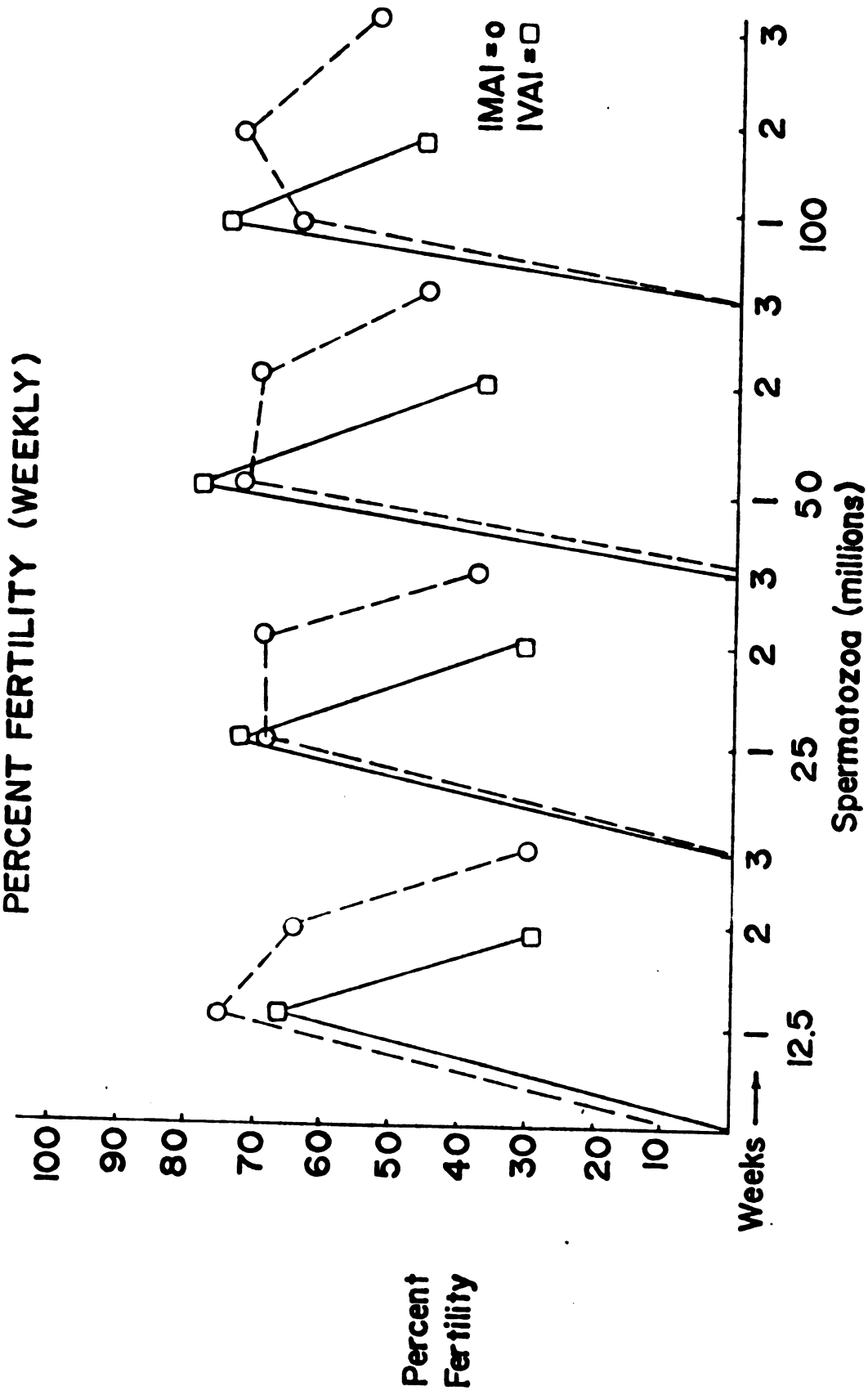


FIGURE 7
ROUTE AND DILUTION INTERACTION
PERCENT FERTILITY (WEEKLY)