ABSTRACT

M.Sc. David M. Bird Renewable Resources

ARTIFICIAL INSEMINATION AND SEMEN PRODUCTION OF THE AMERICAN KESTREL

In order to study the practicability of artificial insemination (AI) in breeding birds of prey, a comparison was made between AI and natural mating in non-imprinted American Kestrels (Falco sparverius). Sixteen pairs were used in 1974, eight of which were naturally mated, and 30 pairs were used in 1975, 12 of which were naturally mated.

The fertility and hatchability of eggs from AI and natural mating were highly comparable. The mean duration of fertility in the females was 8.1 days.

In 1975 the mean duration of semen production for 17 males was 74 days, beginning on March 19. In 17 males, the mean semen volume was 14 μl; the mean sperm concentration, 32,400/mm^3; the mean sperm count per ejaculate, 477,500; the mean motility score, 78%; the mean contamination with epithelial debris and urates, 68%; and the mean semen colour, very pale to pale amber.

The following significant correlations were found: age with semen volume (0.57) and sperm count per ejaculate (0.49); body weight with sperm concentration (0.49) and sperm count per ejaculate (0.62); egg fertility with sperm count per ejaculate (0.65); sperm concentration with sperm count per ejaculate (0.65) and sperm motility (0.59); sperm motility with sperm count per ejaculate (0.67); and per cent successful massages with semen volume (-0.51).

ABREGE

M.Sc. David M. Bird Ressources Renouvelables

INSEMINATION ARTIFICIELLE ET PRODUCTION DE SEMENCE CHEZ LE FAUCON CRECERELLE D'AMERIQUE

Afin d'évaluer l'usage de l'insémination artificielle chez les rapaces, nous avons établi, chez des crécerelles d'Amérique (Falco sparverius), une comparaison entre des accouplements naturels et des accouplements par insémination artificielle (IA). L'étude, en 1974, a porté sur 16 paires, dont 8 accouplées naturellement, et, en 1975, sur 30 paires, dont 12 accouplées naturellement.

Les deux modes d'accouplement ont donné des résultats très semblables du point de vue fertilité et éclosion. La durée moyenne de la fertilité de la femelle a été évaluée à 8.1 jours.

En 1975, la période de production de semence de 17 mâles a duré en moyenne 74 jours, après avoir débuté le 19 mars. Chez ces mâles, nous avons obtenu les données suivantes: volume moyen de l'éjaculat, 14 μl; concentration moyenne de spermatozoïdes, 32,400/mm^3; nombre moyen de spermatozoïdes par éjaculat, 477,500; paramètre moyen de motilité, 78%; taux moyen de contamination par débris épithéliaux ou acide urique, 68%; et couleur de la semence, ambrée très pâle à pâle.

Les corrélations suivantes ont été estimées significatives: l'âge avec le volume de l'éjaculat (0.57); l'âge avec le nombre de spermatozoïdes par éjaculat (0.49); le poids corporel avec la concentration de spermatozoïdes (0.49) et le nombre de spermatozoïdes par éjaculat (0.62); la fertilité avec le nombre de spermatozoïdes par éjaculat (0.65); la concentration de spermatozoïdes avec le nombre de spermatozoïdes par éjaculat (0.65) et avec la motilité (0.59); la motilité avec le nombre de spermatozoïdes par éjaculat (0.67); et le pourcentage de collectes fructueuses avec le volume de semence (-0.51).

ARTIFICIAL INSEMINATION AND SEMEN PRODUCTION OF THE AMERICAN KESTREL

by

David Michael Bird

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science.

Department of Renewable Resources
Macdonald College of McGill University
Montreal

November 1975

This thesis is dedicated to the memory of my father who taught me a great deal about life.

ACKNOWLEDGMENTS

The author is extremely grateful to both Dr. P. C. Laquë and my wife, Toni Bird for their constant help in carrying out this work and in the preparation of the manuscript. Drs. R. B. Buckland and B. Virgo are acknowledged for their contribution of original ideas to this study. The assistance of Professor M. A. Fanous during the statistical analysis is most appreciated.

The writer thanks Alison Bentley, Alison Hackney, Michael Amos, Gregory Weil, Jean Lambert, Cyrus Poirier, Stewart Bentley and many others for their help in caring for the birds and construction of facilities. A note of thanks goes to Walter Kosyanoff, David A. Bird, and Dr. R. D. Baker for photographic assistance and especially to David Hughes who taught me much about the captive management of raptorial birds.

The loan of experimental birds by Montreal Zoological Garden and the Jardin Zoologique d'Orsainville and the provision of food for the birds by Ayerst Laboratories, Merck-Frosst Laboratories and Bio Breeding Laboratories of Canada, Ltd. are gratefully acknowledged.

Finally, the author wishes to thank the Stewart Fund, the McGill Developmental Fund, the Department of Education of Quebec, the White Owl Awards Committee, the World Wildlife Fund (Canada), the National Research Council of Canada

and many private foundations and individuals for their generous financial support without which this project could never have been undertaken.

TABLE OF CONTENTS

LIST OF FIGURES

Figure | Page

LIST OF TABLES

Table | Page

1. INTRODUCTION

Declining world populations of birds of prey, most notably the Peregrine Falcon (Falco peregrinus) and Bald Eagle (Haliaeetus leucocephalus) have been attributed to poisoning and reproductive failure through man's widespread use of organochlorine insecticides (Fyfe et al, 1969; Hickey, 1969; Enderson and Berger, 1970; Temple, 1972). As an emergency conservation measure, many efforts to breed raptors in captivity have been inaugurated throughout the world.

Success in obtaining fertile eggs from many species, however, has been limited and several researchers have turned to artificial insemination as a possible solution (Berry, 1972; Temple, 1972; Corten, 1973; Grier, 1973). Since the above approach was based upon the use of birds sexually imprinted on man, the work reported in this thesis was undertaken to compare the practicability of forced artificial insemination and natural mating in captive non-imprinted birds of prey. The period of semen production and the factors influencing semen production were also examined.

This study is also part of a program at the Macdonald Raptor Research Centre designed to investigate the reproductive physiology of raptorial birds. Due to its abundance in nature, ease of management and willingness

to breed in captivity, the American Kestrel (Falco sparverius) was chosen as the subject for these experiments.

2. REVIEW OF LITERATURE

2.1. Application of Artificial Insemination Techniques

2.1.1. Cooperative and Forced Techniques of Artificial Insemination

The literature concerning artificial insemination in birds of prey is extremely scant. Only recently with the advent of captive breeding to restore dwindling wild stocks has this practice been extended to raptorial birds.

Artificial insemination (AI) in avian species can generally be categorized as either cooperative AI or forced, massage AI. The first method involves the use of imprinted birds conditioned to accept man as a potential breeding mate. Semen is freely ejaculated in a copulatory fashion by a stimulated male onto the handler's appendages. The collected semen is then deposited into the vagina of a receptive imprinted female. No physical restraint is required in either the collection or insemination process. Limited success with this method has been achieved with Red-tailed Hawks (Buteo jamaicensis) (Temple, 1972), American Goshawks (Accipiter gentilis) (Berry, 1972), Golden Eagles (Aquila chrysaëtos) (Grier, 1973) and Prairie Falcons (Falco mexicanus) (L. Boyd, unpublished data).

Forced, massage AI until recently has been mainly

restricted to domesticated birds. To date, the best method for AI of fowls appears to be that perfected by Burrows and Quinn (1935, 1937) and Quinn and Burrows (1936). This relatively inexpensive and harmless method includes the stimulation of the abdomen and vent by physical manipulation to bring about ejaculation and subsequent 'milking' of the bulbous ducts or vasa deferentia. It has since been applied to turkeys (Burrows and Quinn, 1937; Burrows and Marsden, 1938) and successfully adapted for ducks, peacocks, finches, canaries (Bonadonna, 1939), pigeons and doves (Owen, 1941), geese (Johnson, 1954), quail (Wentworth and Mellen, 1963) and pheasants (Smyth, 1968).

With regard to birds of prey, only limited success with this procedure has been attained with Peregrine Falcons (Maatsch and Beyerbach, 1971) and goshawks (Corten, 1973). Recent successes in Holland in obtaining sperm from several species of eagles, falcons and hawks have led Corten (1974) to conclude that the massage technique developed by Burrows and Quinn (1935, 1937) is "suitable for obtaining sperm from all diurnal birds of prey, even under 'forced' conditions".

2.1.2. Time of Insemination

It is generally agreed among raptor researchers that the optimal time for involuntary insemination during the laying sequence is right after an oviposition (Grier et al, 1972; Corten, 1973; Grier, 1973). It has been possible however, to inseminate goshawks successfully

four days prior to egg-laying with cooperative AI (Berry, 1972), and Corten (1973) recommended inseminating goshawks forty-eight hours prior to the first oviposition. In fowl, many researchers agree that the presence of a hard-shelled egg in the oviduct reduces fertility (Moore and Byerly, 1942; Malstrom, 1943; Parker, 1945; Wyne et al, 1959; Bornstein et al, 1960; Smyth, 1968), while others feel that a membranous or soft-shelled egg actually enhances fertility (Malstrom, 1943; Parker, 1945; Johnston and Parker, 1970) as well as hatchability (Parker, 1945). Other reports, however, indicate that the presence of hard-shelled eggs is not detrimental to fertility (Parker and Arscott, 1965; Marks and Lepore, 1965; Lepore and Marks, 1966; Hughes and Parker, 1970; Howarth, 1971. Only one researcher described a decrease in fertility by the presence of an egg in the magnum (Howarth, 1971). Hughes and Parker (1970) feel that posterior pituitary hormones involved in oviposition may be partially responsible for the reduced fertility obtained from insemination near the time of lay.

2.1.3. Onset and Duration of Fertility

Grier et al (1972) suggested that the duration of fertility in birds of prey is at least seven days. Thus they recommended either weekly inseminations or a single insemination after the first oviposition to fertilize the rest of the clutch. The duration of fertility in goshawks is at least four days (Berry, 1972). Grier (1973) obtained from a Golden Eagle one fertile egg laid nine days after AI

and Fessner (1970) obtained in Peregrines a fertile first egg of a second clutch 10 days after AI. Since Grier (1973) suggested that the handling of his bird for the AI might have interfered with ovulation [I suggest that this handling might have interfered with oviposition and not ovulation], it is therefore possible in these latter two cases that the eggs might have been fertilized much earlier and simply held in the uterus for an abnormally long period.

Smyth (1968) summarized the average duration of fertility in chickens to be 10 to 13 days, pheasants, 20.5 days, and turkeys, 40 to 50 days. He then suggested that inseminations should be performed every 7 days in chickens, 10 to 12 days in pheasants, and in turkeys at a four week interval at the beginning of lay and at shorter intervals as laying continued. The duration of fertility in quail is reported to be 4 to 5 days (Wentworth and Mellen, 1963), in geese, 9.7 days (Johnson, 1954; Kinney and Burger, 1960), in ducks, 8 days (Watanabe and Sugimori, 1957), and in pigeons and doves, also 8 days (Owen, 1941).

Sperm storage glands for spermatozoa have been located in the oviduct of chickens (Bobr et al, 1962), turkeys (Verma and Cherms, 1964), and pheasants and Coturnix quails (J. J. Makos III in Smyth, 1968).

No observations have been published on the presence of sperm storage glands in raptors. A report of 500 to 600 copulations for the insemination of one clutch of goshawk eggs in the wild (Holstein, 1941 in Corten, 1973)

led Corten (1973) to suggest the absence of any spermhost glands in this species.

Most of the first eggs after the first insemination are too developed to be fertilized (Smyth, 1968). Both Crew (1926) and Dunn (1927) agreed that fertile chicken eggs are rarely obtained on the first day following mating, but fertility is well established by the second day. Results vary on the time taken by spermatozoa to travel the length of the chicken oviduct. Fifteen minutes (Howarth, 1971) and one hour (Allen and Grigg, 1957) have been observed after intravaginal insemination. Mimura (1941) reported that two to four hours after intravaginal insemination, sperm still had not passed the uterus. Spermatozoa introduced by intrauterine insemination reach the infundibulum in 15 minutes or less (Allen and Grigg, 1957; Donovan et al, 1969).

2.2. Factors Affecting Semen Production

It has become increasingly evident that semen production in avian species is controlled by a combination of factors. In the last five decades, a great deal of research was devoted to determine the relative significance of factors such as seasonal variation, photoperiod, weather, age, weight, nutrition and others.

2.2.1. Influence of age

The age of male American Kestrels should not prove to be overly important in influencing the onset of sperm production in the first year, as one-year old birds of this species

are very capable of fertilizing eggs (Porter and Wiemeyer, 1970). Burrows and Titus (1939) found no great differences in semen production in cocks as compared to cockerels after the latter reached the stage of full production. In fact, it was slightly higher for the cockerels. However, at least two research groups have reported a high percentage of abnormalities in the spermatozoa of young cockerels (Sampson and Warren, 1939; Van Drimmelin, 1951). Conversely, increasing age may have some influence, as Corten (1974) pointed out that one tiercel goshawk produced semen in the latter half of May when, in earlier years, the bird had ceased by that time. Penquite et al (1930) found that White Leghorn males in their second year were less active and produced less sperm with more variability in sperm numbers. Carson et al (1955a) observed less semen production in two-year old turkeys hatched in 1948. Also of interest is the claim by Lorenz and Lerner (1946) that age at first semen production is inherited in both chickens and turkeys.

2.2.2. Effect of body weight

A slight tendency for semen yield to be correlated within species with body weight was noted by Penquite et al (1930), but numerous exceptions were presented. This is in agreement with the observations by Hartmann and Gleichauf (1974), who found that, on the average, the heaviest cocks produced the largest ejaculates and the lowest sperm concentrations. Grier et al (1972) mentioned in their review of AI in birds of prey that all of their birds had heavy body weights at breeding time but, with their small sample

size, they were not able to experiment with semen production.

2.2.3. Individual Variability

Individual variability may play a role in affecting semen production, as Carson et al (1955b) observed considerable variation in the amount of semen produced by different male turkeys. McCartney (1956) on the other hand found no significant differences between toms for semen volume, sperm motility, pH or sperm concentration, but did for total sperm number per ejaculate. Of some interest here is the finding that a direct genetic correlation may exist between semen production in a male fowl and egg production of his sisters and offspring (Jones and Lamoreux 1942 in Van Drimmelin, 1951).

2.2.4. Time of Year

Seasonal production of avian semen under natural lighting conditions has been demonstrated in chickens (Parker and McSpadden, 1943), turkeys (Carson et al, 1955a), and starlings (Sturnus vulgaris) (Bissonnette, 1930 in Bissonnette and Wadlund, 1932). Semen production in chickens has been observed by Parker and McSpadden (1943) to peak in April and May while in turkeys Carson et al (1955a) found maximum production from February until June and July. With regard to the influence of season on semen production, one must exercise caution to avoid confounding this variable with photoperiod, a factor to be discussed in the next section.

The use of AI has only recently made it possible to study semen production in birds of prey. Grier (1973)

reported the duration of semen production in two of his Golden Eagles to be 70 and 111 days, but gave no information on dates of commencement and termination of this production. A goshawk, according to Berry (1972), attempted copulations over 36 and 49 day periods beginning on April 2 and March 22 respectively. Berry added that semen production was closely synchronized with egg-laying. Bird and Laguë (in press) noted copulatory behaviour in captive American Rough-legged Hawks (Buteo lagopus) from May 13 to early July. Corten (1973) collected sperm from his goshawks from March 14 until some time in May. The following year however, the same birds produced no sperm until the second half of April (Corten, 1974).

2.2.5. Influence of Photoperiod

The importance of photoperiod on gonadal cycles in birds has been discussed by Bissonnette (1936), Rowan (1938), Farner (1959), Wolfson (1959) and Schwab (1970). Bissonnette (1931) found that increased daily light periods caused an increase in spermatogenic activity in starlings and Lamoreux (1943) concluded that at least 12 hours of daily light were required to stimulate effectively semen production in fowls. Exposure of adult male chickens to less than natural light did not increase semen volume and number of sperm per ejaculate, but caused some deterioration in quality. Sixteen hours of light however, caused an improvement in semen quality and quantity (Bonadonna and Pozzi, 1955; Bajpai, 1963). In contrast, Parker and McCluskey (1964) found no significant effect on volume or fertilizing capacity

of fowl semen by single daily light periods of 1, 3, 9 or 13 hours. At present there is no available information regarding the effects of photoperiod on semen production in birds of prey, although Willoughby and Cade (1964) showed the importance of this stimulatory factor on egg-laying in American Kestrels.

2.2.6. Effects of Weather

The dependence of sperm production on weather conditions has been little studied. Bissonnette (1931) concluded that temperature and barometric pressure were not controlling factors in conditioning sexual changes during his photoperiod experiments on starlings. Wheeler and Andrews (1943) observed no effects on semen production in fowl by maintaining artificially high or low temperatures. As further evidence, Willoughby and Cade (1964) were able to induce laying of fertile eggs by American Kestrels during the winter months, regardless of temperature, by the manipulation of the photoperiod.

2.2.7. Influence of Management of Males

It has been recommended with pigeons and doves (Owen, 1941), and fowl (Burrows and Quinn, 1937) that males to be used for AI should be kept in separate quarters. Both Owen (1941) and Burrows and Quinn (1937) found that keeping males together in large pens or mated with females resulted in regular ejaculations, but small yields. Conversely, Lorenz et al (1956) found semen production of mated birds not to differ significantly from that of non-mated birds in either volume or concentration. If intervals of

collection exceeded one week however, yields decreased.

Little attention has been paid to the effect of lack of exercise by holding AI males in small quarters. Rowan (1928) concluded from his work on Slate-coloured Juncos (Junco hyemalis) that exercise resulted in testis development in birds. Bissonnette (1931) observed that additional exercise did not increase the spermatogenic activity in male starlings. Neither reported though, on the effects of lack of exercise on spermatogenic activity.

2.2.8. Influence of Nutrition

Grier et al (1972) believe nutrition to be important for semen quality. They recommend for birds of prey undergoing AI a diet of fresh or frozen whole animals, access to direct sunlight and possible vitamin supplements. Although Smyth (1968) reported no conclusive evidence of the effects of Ca and P on semen production, recent work by Molnar et al (1971) and Kovacs (1973) show a marked increase in sperm concentration and sperm viability in ganders fed a high calcium diet. In addition, Kovacs (1973) obtained increased sperm concentration and ejaculate volume with increased dietary phosphorus. Their results differ however, with respect to the effect of the Ca:P ratio, the former observing a decrease in ejaculate volume with a ratio greater than one and the latter believing the ratio not to be of primary importance.

2.2.9. Effect of Frequency of Collection

That the volume of fowl semen produced diminishes with an increase in frequency of collection has been shown

by many workers (Penquite et al, 1930; Sampson and Warren, 1939; Lorenz et al, 1955; McCartney et al, 1958; Nestor and Brown, 1971). Temple in Grier et al (1972) pointed out that he obtained lesser volumes of ejaculate from a Red-tailed Hawk with more frequent collections. Corten (1973) found that daily harvesting of goshawk sperm did not appear to be detrimental to semen quality. Although pigeons can be ejaculated several times in a day, Owen (1941) reported that regular collections more than once a day caused the male to become aspermic. Although male birds usually produce greater amounts of semen after a training period (McCartney, 1956), the above evidence indicates a minimum and a maximum frequency of collection that appears to vary with species. Smyth (1968) concluded that healthy male chickens can be ejaculated three times weekly over extended periods of time without any apparent decrease in volume or fertility. Collections from turkey toms can be made as frequent as two-day to weekly intervals without affecting the yield (Lorenz et al, 1955). In contrast, Nestor and Brown (1971) noted that not two-day but three to four-day intervals resulted in greater semen volume and number of sperm per ejaculate and McCartney et al (1958) found that semen volume decreased gradually as the frequency of collection was increased from 1 to 5 successive days.

2.3. Relationship of Semen Characteristics with Fertility and Hatchability

Much research has been done to correlate semen char-

acteristics with fertility and hatchability. This would hopefully enable breeders to evaluate and predict the reproductive performance of male birds. The characteristics to be discussed in the following paragraphs include motility of spermatozoa, duration of motility under storage conditions, spermatozoa concentration and counts, semen volume, visual appearance, amount of contamination and per cent abnormal spermatozoa.

Grier et al (1972) conclude in their report on AI of raptors that the best semen is "a swarming mass of very active, fully developed motile sperm. Poorer semen is less dense with sperm, contains relatively non-motile or slowly moving sperm, and may contain more abnormally-shaped sperm." With respect to fowl, many researchers have found a positive correlation between motility and fertility (Shaffner and Andrews, 1948; Allen and Champion, 1955; Cooper and Rowell, 1958; McDaniel and Craig, 1959, 1962; Kamar, 1960; Smyth, 1968; Kammerer et al, 1972; Mymrin et al, 1972; Kamar and Razik, 1973). Others have found no evidence of this relationship (McCartney, 1956; Grosse, 1957 in McDaniel and Craig, 1962; Boone and Huston, 1963). Kamar (1960) and Kamar and Razik (1973) further define a relationship between motility and hatchability. The evidence in favour of at least a correlation between motility and fertility have led Cooper and Rowell (1958), McDaniel and Craig (1962), Smyth (1968), and Kammerer et al (1972) to conclude that motility is a relatively effective predictor of fertilizing capacity.

Duration of motility under storage conditions is yet another criteria for evaluating semen quality. It is necessary however, first to determine those conditions under which semen can be stored without excessive impairment. As reported by Warren and Gish (1943) for poultry, Grier et al (1972) recommend a storage temperature of 4.4 to 10.0°C for hawk semen. No mention is made regarding a suitable diluent. Semen from a Golden Eagle stored in a glass vial for up to 3½ hours showed no visible decrease in estimated percent motility (Grier, 1973). Corten (1973) reported good motility in his goshawk semen samples stored for 3 hours at 15°C in Heythusen diluent no. 3. Furthermore, he observed life for as long as 24 hours, but with very low motility. This temperature coincides reasonably well with that reported for fowl by Hunsaker et al (1956). Garren and Shaffner (1952) also recommend gradual temperature changes to avoid shock to spermatozoa.

Some fowl researchers maintain the existence of an association between semen volume and fertility (Kamar, 1960; Kamar and Razik, 1973), but many found no evidence for this relationship (Shaffner and Andrews, 1948; McCartney, 1956; Boone and Huston, 1963; Smyth, 1968; Hartmann and Gleichauf, 1974). Recent work shows no agreement on a correlation between semen volume and hatchability (Mymrin et al, 1972; Kamar and Razik, 1973).

Shaffner and Andrews (1948), McCartney (1956) and Boone and Huston (1963) failed to report any association between concentration of spermatozoa and fertility. In

contrast, several other researchers, the majority in recent years, have recorded a significant correlation in this regard (Cooper and Rowell, 1958; Kamar, 1960; Mymrin et al, 1972; Kamar and Razik, 1973; Hartmann and Gleichauf, 1974). As yet, no evidence exists to discount a possible relationship between concentration of spermatozoa and hatchability (Kamar, 1960; Kamar and Razik, 1973). According to Munro (1938), there exists a minimum number of spermatozoa for fertilization. The lower the concentration of sperm in the semen, the larger is the volume of ejaculate required for an insemination with optimum chance of success.

Grier et al (1972) described hawk semen as a "clearer fluid, appearing somewhat like a drop of concentrated 'lemon juice' which became cloudier with increased numbers of sperm". Corten (1973) reported that goshawk semen is clear watery to milky viscous. In White Leghorn chickens, McDaniel and Craig (1959) found semen appearance scores to be significantly associated with fertility. Later work by these researchers showed no such relation in two strains of chickens (McDaniel and Craig, 1962). Saeki and Brown (1962) found a correlation between colour of turkey semen, numbers of crooked-neck sperm and decreasing fertility. Brown and Graham (1971) rated visually the appearance of turkey semen with scores from 1 to 3 based subjectively on colour, volume and density. The discovery of a highly significant correlation between semen quality and fertility led these researchers to claim that visual ratings are acceptable for selecting high quality semen.

With respect to sperm counts, Sampson and Warren (1939) found indications that these are not a very accurate means of detection of males with poor fertility. Recent work by Hartmann and Gleichauf (1974) also showed the non-existence of a relationship between sperm counts and fertility.

Corten (1973) usually found in goshawk semen contamination by faeces, urine, blood and various other undescribed exudates from the cloaca and pointed out that too much of these contaminants can be detrimental to the survival of the spermatozoa. In chickens, as little as 1% fecal contamination is apparently deleterious to motility and vigour of spermatozoa as well as fertility (Boone and Hughes, 1970). Immediate insemination however, of semen contaminated with white chalky urates likely does not reduce the fertilizing capacity of the spermatozoa (Smyth, 1968).

To enable an assessment of percentage abnormal spermatozoa in predicting semen values, one must have prior knowledge of the differences between normal and abnormal spermatozoa. Heads of falcon spermatozoa have been described by Corten (1973, 1974) to be somewhat rod-shaped with the acrosome broader and less pointed. The spermatozoa from falcons are apparently the same size as those of goshawks; the heads being 12.5 μm, the tails 50.0 μm, and the total length ranging from 62.5 to 70.0 μm (Corten, 1974). No descriptions of abnormal spermatozoa have been reported for raptorial birds. The major abnormalities found in

poultry sperm are absence of tails, coiled tails, blunted heads with less frequent occurrences of short and broken heads, small immature sizes and extreme clumping of their numbers (Sampson and Warren, 1939). Bajpai (1963) also noted abnormally elongated heads. A negative correlation between percent abnormal spermatozoa and fertility has been established by Allen and Champion (1955) and Saeki (1960), while Kamar (1960) did not find such a relationship.

Other aspects of the relationships between the aforementioned semen characteristics should be included to elucidate further upon the importance of these characteristics. McDaniel and Craig (1959) and Mymrin et al (1972) found a significant positive correlation between motility of fowl spermatozoa and semen volume, as well as between motility and concentration of spermatozoa. A negative correlation between motility of spermatozoa and the percent abnormal spermatozoa in fowl has been recorded by Allen and Champion (1955). Finally, a relationship between motility and visual appearance subjectively based on colour, density and volume has been reported in turkeys (Brown and Graham, 1971). A positive correlation (McDaniel and Craig, 1959; Mymrin et al, 1972) as well as a negative one (Carson et al, 1955b) have been shown between semen volume and concentration of spermatozoa in poultry. Nishiyama et al (1971) found concentration of fowl spermatozoa to have a highly significant effect on sperm lifespan, lifespan and motility being greatest at low concentrations and lowest at high concentrations.

3. SPECIFIC STATEMENT OF PROBLEM

The increased interest in the practice of artificial insemination by raptor breeders to improve the reproductive efficiency of their birds has met with many unanswered problems. These problems must be solved in order to increase the efficiency of artificial breeding and to make sound recommendations to breeders employing this practice in their breeding program.

The objects of this thesis are:

1. To describe a method of semen collection and insemination in small falcons.
2. To present data to assess the value of the above techniques.
3. To determine the period of semen production in male American Kestrels and factors affecting semen production.
4. To determine the duration of fertility in female American Kestrels.
5. To relate several semen characteristics with fertility and hatchability.

4. MATERIALS AND METHODS

4.1. Source of Birds

The original colony was established in 1973 by obtaining 10 pairs of kestrels from zoos and nests in the Montreal area. In 1974 the colony was extended to 16 pairs consisting of the original 10 pairs, their progeny and several orphaned nestlings from nests in the vicinity of Montreal. The progeny from the 16 pairs, both hand-reared and parent-raised, and several more orphaned birds were incorporated into the colony to increase the number to 30 pairs in 1975. To prevent inbreeding, at no time prior to and during the experimentation were related matings permitted. Otherwise, all birds were chosen randomly for mating, such that each pair consisted of a yearling and an older bird.

4.2. Housing and Management

In 1974, with the exception of a brief period of visual isolation in cages 0.5 x 0.3 x 0.3 m (L x W x H) for some males, all males to be used for AI were tethered on blocks in falconer's fashion in a sanded room 6.6 x 6.6 x 2.5 m (Fig. 1). In 1975, all males were kept in Aspenite box cages measuring 0.7 x 0.7 x 0.7 m with .25 cm nylon mesh on one side (Fig. 2). For the most part they were kept three to a cage. This was decreased to two per cage with the addition of two more cages on May 17.

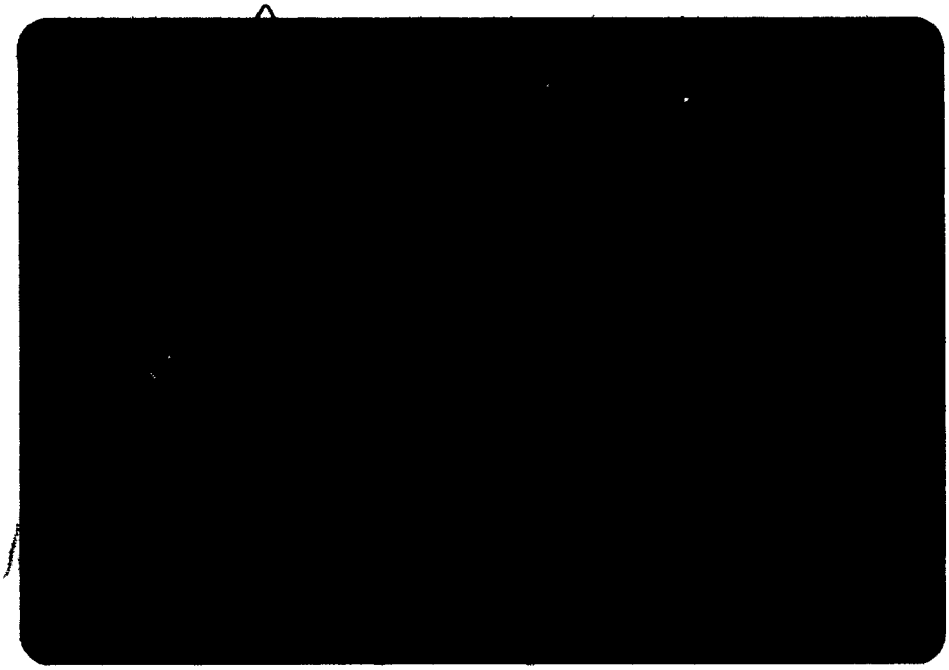


Figure 1. AI kestrel males tethered in falconer's fashion in 1974.

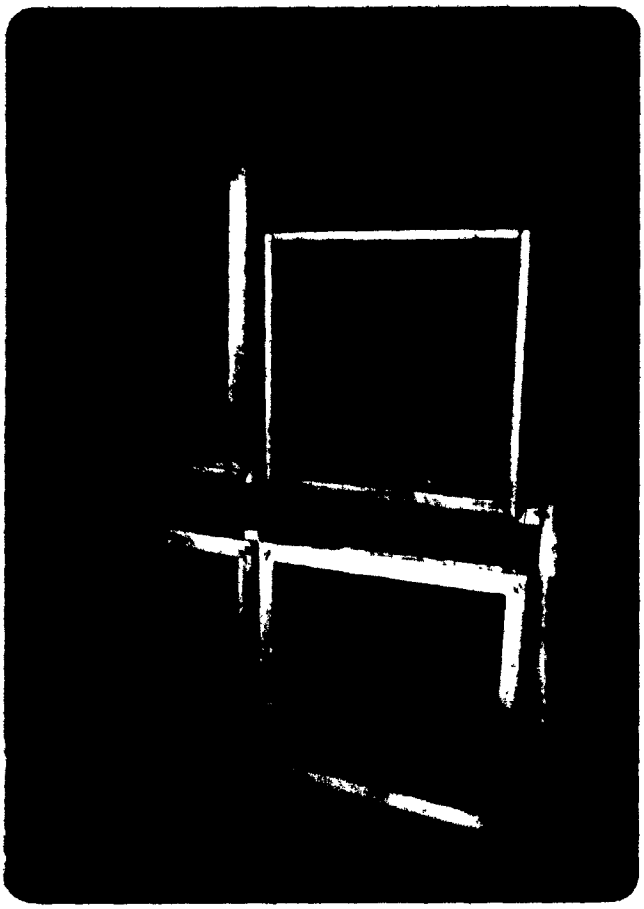


Figure 2. Box cages which housed the AI kestrel males in 1975.

Paired birds and single AI females were caged under one of two different conditions, that of visual isolation or exposure to other birds. Visual isolation was achieved by placing the bird(s) in pens 2.7 x 1.5 x 2.5 m with insulated Aspenite walls, a window of 2.5 cm wire grid measuring 1.5 x 1.3 m (W x H) and a sanded floor (Fig. 3). Exposure to other birds was achieved in a cluster of eight pens of similar size as those above built into a sanded room 6.6 x 6.6 x 2.5 m (Fig. 4). The main difference here was that all inner walls consisted of .25 cm nylon mesh. All pens contained suitable perches, a small shelf and one nest-box measuring 25.4 x 25.4 x 45.7 cm with a hole .76 cm in diameter placed 2 m above floor. All birds were kept under natural lighting conditions.

The diet consisted mainly of frozen-thawed day-old chicks with occasional feedings of frozen-thawed adult mice. In 1975 only, the day-old chicks were rolled in bonemeal to supplement calcium and phosphorus in all birds and every five days SA-37 (Rogar-STB Division of BTI Products Inc., Montreal, Quebec) vitamin supplements were also added. Bath-water was supplied regularly to all birds in the warmer months.

Just prior to their introduction into their respective pens, all birds were weighed. In 1974 and 1975, the males were placed in their pens in the first week of March and the females introduced one week later.

4.3. Experimental Design

In 1974, 32 birds were used in the studies: 8 control or naturally mated (NM) pairs, and 8 AI females and 8 AI

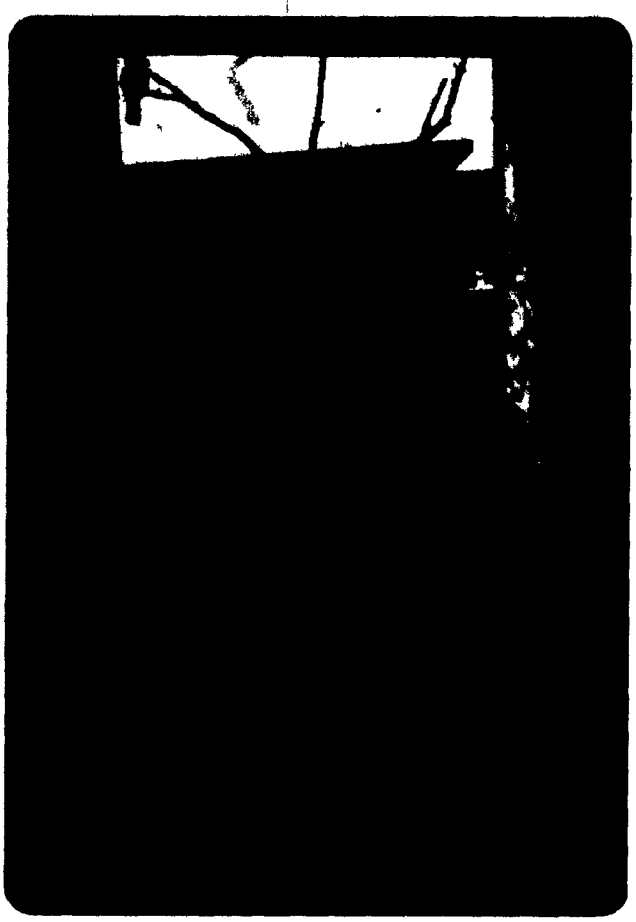


Figure 3. An isolated pen which housed either a kestrel pair or a single AI female.

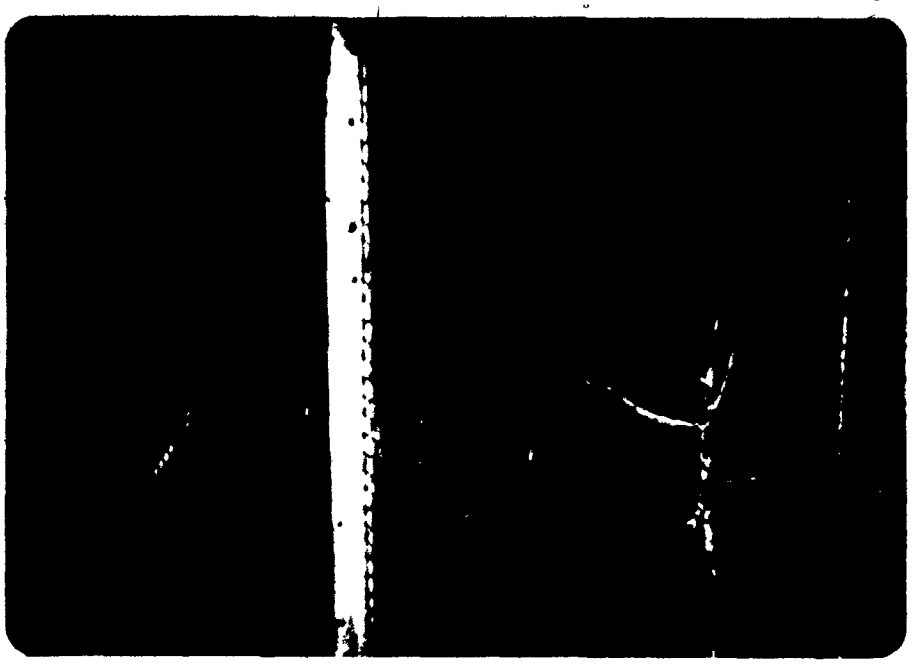


Figure 4. Several exposed pens which housed kestrel pairs and single AI females.

males. One-half of the control pairs and one-half of the AI females were kept in isolated quarters and the rest in the exposed cluster pens.

In 1975 the sample size was increased to 60 birds, 24 of which made up 12 NM pairs. Six pairs were assigned to isolated pens and the remaining six to exposed pens. The remaining 36 birds were divided as 18 AI females and 18 AI males. Eight of the AI females were placed in isolated pens and 10 assigned to exposed pens. The first 12 AI females to lay eggs were included in the comparative study between AI and NM, while any other single females laying eggs were tested for duration of fertility.

4.4. Incubation Practices

Nestboxes were examined once a day prior to the onset of egg-laying after which they were inspected three times a day. Records were kept of laying times. The date laid and the pen number were marked on all eggs with a graphite pencil. In 1974, the whole first clutch of all laying females was removed generally on the fourth day following the last egg laid. The second clutch was left for natural incubation and was only removed upon failure of the female to incubate. This procedure was followed in 1975 with the exception that the eggs of the second clutch of all AI females were removed one by one leaving one in the nest for duration of fertility experiments. Also treated in this manner was the first and only clutch of the thirteenth laying female.

In 1974, the eggs collected were immediately placed

in a forced air Marsh Farms Roll-X (College Pets, Poultry and Garden Supplies, Toronto, Ontario) incubator kept at 38°C and a 53-59% relative humidity (Fig. 5). The eggs were turned automatically every hour and were placed upon pipping into a hatcher kept at 37°C and about 70% relative humidity. The hatcher consisted of a Marsh Farms Roll-X incubator with inverted grids. The chicks were all hand-raised.

Brooding of hand-raised chicks was achieved through use of a styrofoam cooler with heating tape, thermostat and water pan (Fig. 6). Temperatures were controlled to maintain the chicks' comfort, usually beginning at 35°C after hatching and decreasing gradually to room temperature by 2 weeks of age.

The chicks were fed four times daily by offering chopped day-old mice to them with blunt forceps. As the chicks grew older, this food was replaced by day-old chicks and adult mice mashed whole in a blender.

In 1975 a second incubator and a hatcher of the same type were obtained. The same environmental conditions were used except that the humidity was always just above 60% in the new incubator due to its characteristics. The chicks were also hand-raised but when possible, some were introduced for fostering under parents whose eggs failed to hatch. No attempts were made to control temperature or humidity in the incubator room.

4.5. Technique of Collection and Insemination

4.5.1. Materials and Preparation

The insemination tube consists of 10 cm of no. 4

Figure 5. Kestrel eggs placed over the water reservoirs in a Marsh Farms Roll-X incubator with an automatic turner.

Figure 6. Brooding of hand-reared kestrel chicks in a styrofoam cooler with heating tape, thermostat and water pan. The corrals allow for chick identification prior to marking.

medical grade vinyl tubing (I.D. x O.D., 0.044" x 0.064", Becton, Dickinson and Company, Rutherford, New Jersey, U.S.A.) attached to an 18 gauge blunted needle and a 1 ml syringe. Silastic tubing was found to be too soft and pliable and polyethylene tubing, too stiff.

A phosphate buffer (16.34 gm Na_2HPO_4, 5.16 gm $NaH_2PO_4 \cdot H_2O$ per litre; pH 7.2) (Wilcox, 1958) was used in our experiments not as a diluent, but as a solution to wash away all the semen from the tube into the oviduct. To achieve this washing, the semen collector first draws into the syringe about 0.1 ml of air in order to be able to push out the whole volume of fluids at the time of insemination and then enough buffer to fill about 1 cm of the insemination tube. This buffer is drawn up slightly in the tube so that a small air bubble will form between it and the semen to be collected as described later. This precaution prevents the spreading of the semen in the tube.

Sterilization of the insemination tube and the buffer was not done but cleanliness was maintained throughout. The buffer which may be kept at either 4°C or frozen for a breeding season was stored in small aliquots in well stoppered tubes. A partially used aliquot was discarded rather than restored.

In the experimental program, no pooling of semen was done in order to pedigree the chicks. For the same reason, a clean insemination tube was used for each insemination. Smyth (1968) cites the danger of contamination in pooled semen by feces or urates from a contrary male and

the possibility of spreading disease by the use of artificial insemination in poultry.

The males were 'worked' 3 times a week for approximately 5 weeks before insemination was begun. This training consisted of handling the birds and attempting to ejaculate them.

4.5.2. Collection of Semen

The method of semen collection follows, in principles, the massage method described by Burrows and Quinn (1937). At first, three persons were needed for the procedure. One held the bird's feet and head, another ejaculated the bird and the third collected the semen in the insemination tube. But with practice, it was found that the semen collection could be accomplished by two persons in as much as the bird holder would wear a protective but flexible pair of gloves which gives him freedom to ejaculate the bird. The thumb and two forefingers of the glove to be worn on the massaging hand, depending on the operator's preference, were cut off halfway down. Thus the glove uncovers only the finger tips needed for the sensitive massaging process.

The massager grasps the bird's thighs together with one hand and although not necessary, rests the bird with its breast on a block. The wings are left free to flap to stimulate natural copulation. The feathers surrounding the vent area do not have to be plucked but just pushed aside. The complete semen collection can be divided in three steps: massaging, milking, and collecting the semen in the insemination tube.

The massager's free hand with the exposed fingertips

is placed palm down on the bird's back, the thumb and fingers on opposite sides (Fig. 7). Gentle but firm strokes are made towards the pygostyle area. At the same time, the digits massage the soft lateral parts of the abdomen below the pelvic regions. When the papillae are everted from the cloaca, the tail is pushed upwards by the back of the free hand and the milking process begins with the thumb and forefinger on opposite sides of the cloaca, frontally of the protruding papillae (Fig. 8). In addition, slight pressure is placed just below the cloaca with the other thumb. This is important as it was found that ommission of this step sometimes resulted in the formation of a sore caused by the thumb of the massaging hand. With reasonably firm pressure, these digits squeeze or 'milk' the semen from the papillae. Any urates present can be wiped away with a finger. Squeezing too hard or for prolonged periods may result in hemorrhaging in which case semen collection should be postponed for at least two days.

The semen usually flows into the groove formed by the union of the thumb and forefinger. In some cases, the thumb of the hand holding the bird's leg was lifted up to catch the semen on the thumbnail. In only a few instances was the semen forcefully expelled into the air from the papillae.

Since the yield of semen in kestrels is very small, it takes considerable skill to collect most of it in the insemination tube. It is preferable during the aspiration of the semen in the tube to keep the semen near the tip to

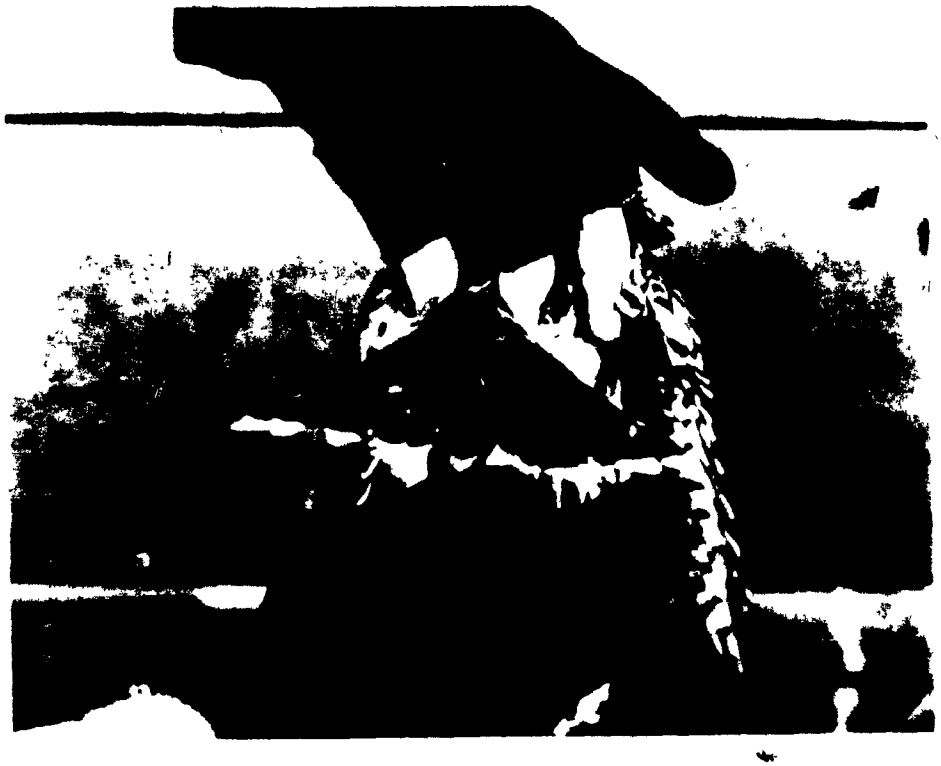


Figure 7. Grasping of the bird's thighs by one gloved hand, while the other hand massages the soft lateral parts of the abdomen.

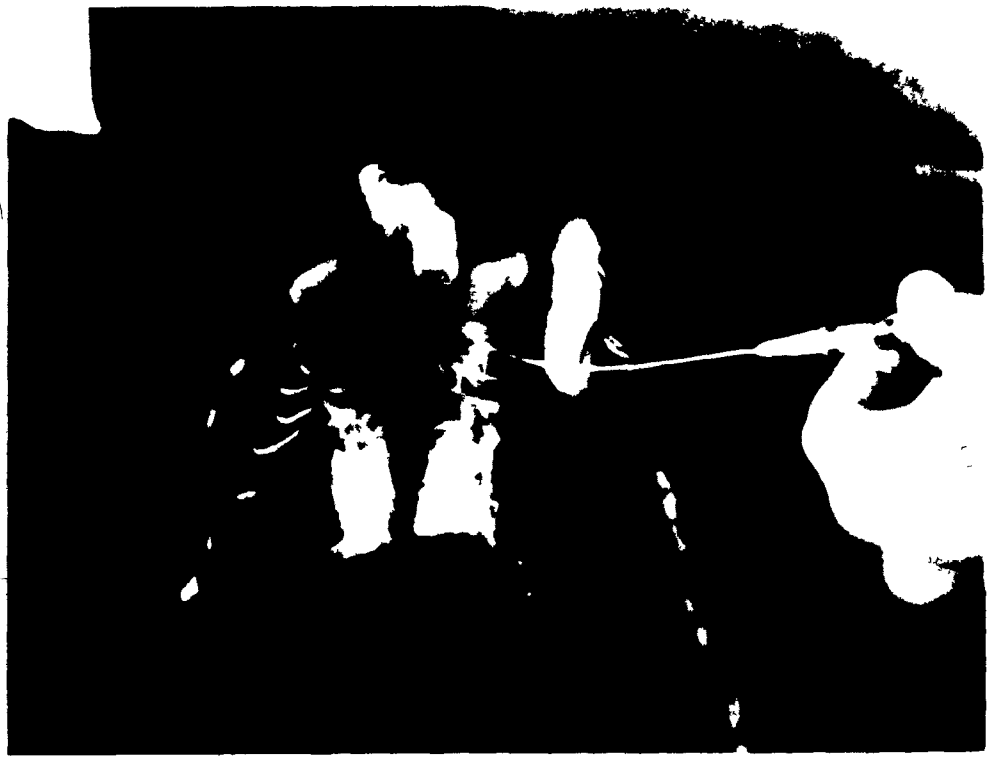


Figure 8. Milking of the semen from the cloacal papillae and the subsequent collection of the semen in the insemination tube.

prevent loss of semen on the walls. The process of milking can be repeated until the yield stops.

No precautions were taken to maintain the semen temperature between the time of collection and insemination However, the samples were never held for more than 10 minutes at ambient temperature and, at least in chickens, spermatozoa do not lose their fertilizing capacities after rapid cooling for up to 10 minutes (De Silva, 1963).

4.5.3. Insemination of the Female

The females were inseminated as soon as possible after an oviposition and only once between subsequent ovipositions.

Several attempts to evert females prior to the egg-laying period resulted in failure. However, by immediately removing the first egg laid, which is obviously infertile, the bird generally does continue to lay a clutch of a normal number.

The technique used to inseminate the females is adapted from that of Quinn and Burrows (1936). One person holds the bird's thighs together in one hand and controls its wings and head with the other hand to prevent flapping wings from knocking the semen out of the insemination tube. The bird is tipped forward to expose the cloacal region. Once the bird is properly secured, the thumb of the hand holding the legs can be freed to place slight pressure on the abdomen just below the cloacal region. The second person, while holding the syringe in one hand and away from the bird, places his thumb and forefinger of the other hand

on each side of the cloaca (Fig. 9). The cloacal region is then massaged by a gentle, caudal to frontal stroking. Enough pressure and massage are applied to evert the oviduct which appears as a deep-red fleshy mass with a slightly visible opening on the left side. This functional left oviduct can be located quickly only with a practiced eye. Any urates ejected can be wiped away by the operator's finger.

The insemination tube is placed with careful manipulation about 1 to 2 cm deep into the left oviduct (Fig. 10). In this action, care must be taken not to puncture any soft-shelled egg if present nor to injure the bird. The presence of an egg in the oviduct may be determined by external palpation of the abdomen when the bird is captured.

As the semen is deposited into the oviduct the pressure of the thumb on the abdomen should be gradually released. The tube is withdrawn when empty. When the oviduct has fully retracted, the bird is released.

4.6. Semen Production

Beginning the first week of March in 1974 and 1975, the AI males were 'worked' three times a week as stated earlier. This training was discontinued only when the semen was needed for insemination but recontinued after inseminations were completed. Late in the laying season the AI males were ejaculated once a week to determine termination of semen production. For the same reason, the paired males were 'worked' once a week, but only after

Figure 9. Massaging of the cloaca of the female to evert the oviduct, i.e. expose her vagina, for insemination. Note the thumb of the operator applying abdominal pressure.

Figure 10. Placing of the insemination tube about 2 cm into the left oviduct to deposit the semen upon successful eversion of the oviduct.

egg-laying completely ceased. In 1975 only, 7 extra males flying loose in a large pen with three females were also 'worked' weekly late in the season to determine semen production under this method of management.

Studies were conducted on the influences of age and body weight of the AI males on per cent successful massages, semen volume and colour, concentration and motility of spermatozoa, sperm count per ejaculate, and per cent contamination in 1975.

Although the AI males were kept on a natural lighting regime, an attempt was made to correlate time of year and increasing day-length with semen production. This was accomplished by referring to a chart of standard time zones for the daylight changes at 46° latitude.

The effects of weather, i.e. temperature, barometric pressure, per cent possible daily sunshine, on the beginning and ending of semen production were studied. The effects of weather conditions on semen characteristics were also studied. Records of weather conditions for 1974 and 1975 were made available by the McGill University weather station situated at Macdonald College.

Where possible, any effects of frequency of collection and nutrition on semen production were recorded.

4.7. Relationship of Semen Characteristics with Fertility and Hatchability

4.7.1. Fertility and Hatchability Ratios

Eggs were classified as fertile or infertile by candling at various stages of development. All eggs

classified infertile were stored at 4.4°C and later broken and examined microscopically for evidence of embryo development.

The term "per cent fertile" refers to the percentage of fertile eggs relative to the total number laid. The term "per cent fertile from AI" is the percentage of fertile eggs relative to the number of potentially fertilized eggs. The percentage of eggs which hatched relative to the total number fertile was termed "per cent hatchability".

"Duration of fertility" was measured as the number of days a hen was fertile, i.e. from the first day after insemination until the last fertile egg was laid. "Per cent fertility while fertile" is the percentage of fertile eggs relative to the total number of eggs laid during the duration of fertility.

"Per cent successful massages" refers to the percentage of massages resulting in collections of measurable semen volume relative to the total number of attempts during the period of actual semen production.

4.7.2. Analysis of Semen Characteristics

Any relationships among per cent successful massages, semen volume, concentration of spermatozoa, sperm count per ejaculate volume, motility of spermatozoa, semen appearance and per cent contamination were tested for significance. With the exception of per cent successful massages, all of the above characteristics were tested for possible correlations with fertility and hatchability.

4.7.3. Determination of Semen Characteristics

On every semen sample collected, the following six characteristics were analyzed: semen appearance, semen volume, concentration of spermatozoa, sperm count per ejaculate volume, motility of spermatozoa, and per cent contamination.

4.7.3.1. Semen Appearance

The appearance of the semen sample was classified into one of six different categories. These were denoted as: 0 - opaque

1 - clear

2 - very pale amber

3 - pale amber

4 - amber

5 - deep amber

4.7.3.2. Semen Volume

Semen volume was calculated by first measuring the length of tubing filled by the semen. The pre-determination of the volume of fluid occupying 1 cm of this tubing facilitated simple calculation of the unknown volumes.

4.7.3.3. Concentration of Spermatozoa

The concentration of spermatozoa was determined by means of an Improved Neubauer hemacytometer. Although the small size of the spermatozoa and heavy debris cover in the semen did not permit exact counts after immobilization of spermatozoa with ethanol, their slower movement and smaller numbers allowed an accurate estimate to be made of live, as well as any observed immobilized spermatozoa. As dilution was not feasible due to the small amount of semen obtained,

a revised method of counting on the grid was employed to save time. The same five groups of sixteen small cells used to count red blood cells were involved, but only spermatozoa in the four corner cells of each group were counted. The calculations were made as follows:

$$\frac{\text{No. sperm counted}}{\text{No. squares counted}} \times \frac{1}{\text{area of square}} \times \frac{1}{\text{depth of chamber}}$$

$$= \frac{\text{No. sperm counted}}{20} \times 400 \times 10$$

After five counts were obtained on each AI male, the above method was discarded in favour of randomly choosing several small cells and estimating the mean numbers of spermatozoa per cell. The calculations were then made as follows:

number of spermatozoa x 400 x 10

4.7.3.4. Sperm Count per Ejaculate Volume

This was simply obtained by multiplying the concentration of spermatozoa per cubic millimeter with the measured volume of semen.

4.7.3.5. Motility of Spermatozoa

All samples of undiluted semen were given motility ratings from 0 to 5 immediately upon placing the fresh semen on a hemacytometer. A description of motility scoring was as follows:

0 - no motility discernable

1 - 1-20% sperm are motile. May or may not be progressive motility. Mostly weak and oscillatory. May be low numbers of spermatozoa.

2 - 20-40% sperm motile. Mostly progressive, but some

immobilized by debris and still exhibiting life signs. At least 40% totally inactive. May be low numbers of spermatozoa.

3 - 40-60% sperm motile. Fairly vigorous and progressive motility. 20 to 30% totally inactive. 20 to 30% immobilized by debris, but still exhibiting life signs. Good numbers of spermatozoa.

4 - 60-80% sperm showing progressive motility. 10 to 20% immobilized by debris, but showing vigorous life signs. 10 to 20% totally inactive. Many spermatozoa.

5 - 80-100% showing vigorous, progressive motility. Less than 10% immobilized by debris and less than 10% totally inactive. Many spermatozoa.

4.7.3.6. Duration of Motility

When samples were available, the duration of motility of semen held at both room and refrigerated conditions, i.e. 4.4 to 10°C, as well as mixed with Wilcox phosphate buffer was examined. Pooled samples were stored in glass tubes and the refrigerated samples were placed directly into the refrigerator.

4.7.3.7. Per Cent Contamination

The "per cent contamination" was measured by expressing the amount of area on a hemacytometer covered by debris as a percentage of a microscopic field 400X in magnification.

4.7.4. Statistical Analysis

The analyses of variance and simple parametric

correlations were performed according to Steel and Torrie (1960). Non-parametric simple correlations were done in accordance with Siegel (1956).

5. EXPERIMENTAL RESULTS

5.1. Egg Production, Fertility and Hatchability

The results shown in Table 1 justify the assumption in these experiments that age of the females would not affect whether or not they laid eggs. There was no appreciable difference between the percentages of yearlings laying eggs and those two years or older laying eggs.

Although no significant ($P>0.05$) effects of pen design, i.e. isolated vs. exposed, or mating types, i.e. AI vs. NM, on per cent females laying were noted in Appendix Table 1, a coefficient of variability of 28.6 per cent indicates only a moderate precision, likely due to a small sample size. As indicated in Table 2, 15.7 per cent more AI females than NM females laid eggs in exposed pens. More noteworthy is the fact that in isolated quarters, all NM females laid eggs while only 60 per cent AI females laid eggs under these conditions.

With respect to NM pairs only, the pen design had no outstanding effects on fertility of either first or second clutches (Table 3). However, the hatchability of artificially and naturally incubated eggs laid under isolated conditions was 90.3 and 84.6 per cent, respectively, whereas in those eggs laid in exposed pens, the hatchability was only 47.6 and 21.7 per cent, respectively, as seen in Table 3.

No significant ($P>0.05$) differences (Appendix Table

TABLE 1

The Effect of Age on First Laying in Female Kestrels

	Age	
	yearlings	2nd year or older
Total number of females	20	23
Number of laying females	16	19
Per cent laying females	80	82.6

TABLE 2

Per Cent Female Kestrels Laying in Exposed Pens and Isolated Pens

	Exposed Pens		Isolated Pens	
	AI	NM	AI	NM
Total number	14	10	10	10
Number of laying females	12	7	6	10
Per cent laying females	85.7	70	60	100
	mean 77.9		mean 80	

TABLE 3

Fertility and Hatchability in NM Kestrel Pairs
In Isolated and Exposed Pens

		Exposed Pens	Isolated Pens
Per Cent Fertility	1st clutch	56.8 (21/37)*	62.0 (31/50)
	2nd clutch	76.7 (23/30)	63.4 (26/41)
Per Cent Hatch.	artificial incubation	47.6 (10/21)**	90.3 (28/31)
	natural incubation	21.7 (5/23)	84.6 (22/26)

* 21 out of 37 eggs were classified as fertile
** 10 out of 21 fertile eggs hatched

2) were encountered between years, first and second clutches or mating types with respect to the percentage laying females producing fertile eggs (Table 4). A coefficient of variability of 15.2 per cent indicates a satisfactory precision.

The fertility and hatchability of individual AI females are presented in Table 5. The overall mean per cent fertility from AI for both years is 76.4 ranging from 25 to 100 per cent. The overall mean per cent hatchability for both years is 55.5, ranging from 0 to 100 per cent. The per cent fertility of eggs laid by AI females with respect to their position in the clutch is illustrated by Figure 11. The second egg, which was the first egg laid after the first insemination, was fertilized less than half the time, while the highest fertility, 90.9 per cent, was found in the sixth egg laid.

The fertility of AI and NM females is shown in Table 6, with the corresponding statistical analysis in Appendix Table 3. In either mating type no significant ($P>0.05$) differences were located between the fertilities and the fertilities from AI. The corresponding coefficients of variability, 35.8 and 29.7 per cent respectively, indicate a lack of precision and therefore, the differences in the figures in Table 6 are emphasized. Both fertility and the fertility from AI rose from 1974 to 1975 by 29.0 and 19.7 per cent, respectively.

Both per cent fertility and per cent fertility from AI of the AI females in 1975 compared extremely well with the per cent fertility of the first and second clutches of

TABLE 4

Per Cent Laying Female Kestrels Producing Fertile Eggs

Year	AI Females		NM Females	
	1st clutch	2nd clutch	1st clutch	2nd clutch
1974	100 (5/5)*	50 (2/4)	80 (4/5)	80 (4/5)
1975	100 (13/13)	89 (8/9)	67 (8/12)	83 (10/12)
Mean	100	70	73	82

* 5 out of 5 females produced fertile eggs

TABLE 5

Fertility and Hatchability of Individual AI Female Kestrels

Year	Bird No.	Laying Sequence After First and Subsequent Inseminations	Per Cent Fertility From AI	Per Cent Hatch.
1974	760	AI-●-O--O-O-X--	25	100
	762	AI--O-O-X-O-X--	40	100
	799	AI-X--X-X-X--X-	100	100
	792	AI-X-X--O------	66	50
	798	AI-●---O--X-X-X	75	100
1975	771	AI-O-X-X-X-X---	80	0
	783	AI-●-X-X-B-----	100	0
	789	AI-O-X--X-X----	75	66
	792	AI-X-O-X-X-X---	80	75
	764	AI--X-X--O-X---	75	0
	798	AI-X--X--X-O-O-	60	66
	40	AI-●-X-X-X-----	100	0
	22	AI--O-X--X-X--X	80	100
	19	AI-X-X--X-X--X-	100	60
	41	AI-●-X-O-X-----	66	0
	16	AI-X-X--X-O----	75	100
	60	AI-●-X-X--X--X-	100	25
		Mean	76	55

X = fertile egg O = infertile egg B = egg broken

- = no egg laid

● these eggs were too far developed to be fertilized by the first insemination

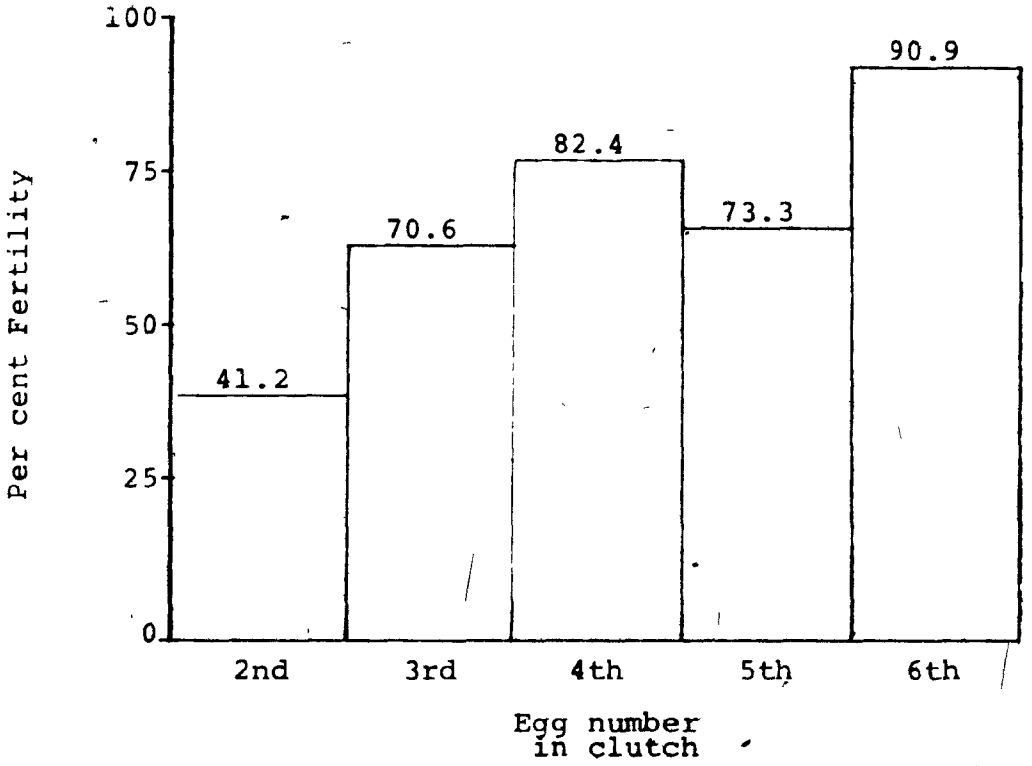


Figure 11. Per cent fertility of eggs laid by AI kestrel females with respect to their position in the clutch.

TABLE 6

Comparison of Fertility Between AI and NM Female Kestrels

Treatment	Year	No. Females Laying		No. Eggs	No. Eggs Laid After AI*	No. Fertile	Per Cent Fertile	Per Cent** Fertile From AI
NM	1974	5/8	1st clutch	26		20	76.9	
			2nd clutch	22		18	81.8	
	1975	12/12	1st clutch	61		27	44.3	
			2nd clutch	49		31	63.3	
			Total	158		96	Mean 60.8	
AI	1974	5/8		28	21	13	46.4	61.9
	1975	12/12		65	49	40	75.4	81.6
			Total	93	70	53	Mean 57	75.7

* The figures in this column exclude those eggs too far developed to be fertilized from the first insemination.

** "per cent fertile from AI" is the percentage of fertile eggs relative to the number of potentially fertilized eggs.

NM females in either year. While the overall per cent fertility in either mating type was very close, the overall fertility from AI ranked 14.9 per cent higher than the overall fertility in the NM group. A considerable decrease in fertility from 1974 to 1975 of the first and second clutches is noted. An increase of fertility in NM females from first to second clutches in both years is also observed.

No significant ($P>0.05$) differences in the hatchability of eggs from AI and NM females under natural or artificial incubation (Table 7) were noted in Appendix Table 4. A coefficient of variability of 14.2 per cent represents a reasonably high precision. One should also note in Table 7 the substantial decrease in hatchability in all three treatments from 1974 to 1975, particularly in the case of eggs produced by AI females and artificially incubated where the decrease is greater than 50 per cent.

The fledging rate of both hand-reared and parent-raised birds was fairly high. Except for one chick born with a defect, mortality among chicks resulted mostly from accidental causes. Over the two years, 25 of 27 chicks were parent-raised for a fledging rate of 92.6 per cent. During the same period, 63 of 72 chicks were hand-reared for a fledging rate of 87.5 per cent; 32 of these were produced by AI. In addition, for experimentation and for the reduction of man-hours consumed by hand-rearing, 11 AI chicks in 1975 were fostered under parents which either had infertile eggs or only one chick. Of these, 7 survived. In all of the chicks hatched in both years, the sex ratio was very

TABLE 7

Hatchability of Fertile Eggs of AI and NM Female Kestrels

Mating	Incubation Method	Year	No. Fertile Eggs Hatched	Per Cent Hatchability
AI	Artificial	1974	18/19	94.7
		1975	18/40	45.0
		Total	36/59	Mean 61.0
NM	Artificial	1974	19/20	95.0
		1975	19/26	73.1
		Total	38/46	Mean 82.6
NM	Natural	1974	13/18	72.2
		1975	14/26	53.8
		Total	27/44	Mean 61.4

nearly one to one.

5.2. Duration of Fertility

The results on the duration of fertility of individual AI females in 1975 are presented in Table 8. The mean duration of fertility was 8.1 days, ranging from 4 to 12 days. The mean per cent fertility from AI was 48.4, ranging from 10 to 100. The per cent fertility while fertile ranged from 50 to 100 with a mean of 70.8. A low per cent hatchability was again encountered, ranging from 0 to 100 with a mean of 42.7.

The Spearman Rank Correlation test (Appendix Table 5) revealed no significant ($P>0.05$) relationships between semen characteristics and the duration of fertility, or the fertility and hatchability associated with the duration of fertility.

5.3. Semen Production

There is no appreciable difference in the per cent successful massages between 1974 and 1975. A mean of 74.2 per cent was obtained over the two years (Table 9).

Table 10 summarizes the percentages of collections yielding semen, urates, blood and nil. The percentage of collections resulting in semen alone was greatest in the months of April and May, dropping considerably in June. The percentage of collections yielding urates however, was greatest in March, declining to less than half in the remaining months. The presence of blood in the collections was prevalent only in March and almost nil in April, May and June. The per cent collections yielding nil in June

TABLE 8

Duration of Fertility of Individual AI Female Kestrels in 1975

Hen No.	AI Egg-laying Sequence	Duration of Fertility (days)	Per Cent Fertile From AI	Per Cent ** Fertile While Fertile	Per Cent Hatchability
33	AI-O-X--O--O-O--O-O-O-O--O-O---	4	10.0	50.0	0.0
798	AI-O-X-X-O-O-O--O----------	6	28.6	66.7	100.0
16	AI--------O-X-O-O-O--O-O--O---O	11	11.1	50.0	100.0
792	AI-O-X-X-O-O-X--O--O---O-O-O---	12	27.3	50.0	0.0
764	AI--X-X-X--X-O----------	10	80.0	100.0	25.0
41	AI--X-X-O-O----------	5	50.0	100.0	50.0
789	AI-O--X-X-X----------	9	100.0	75.0	0.0
783	AI-X-X-O--------X----------	8*	75.0	75.0	66.7
	Mean	8.1	48.4	70.8	42.7

X = fertile egg O = infertile egg - = no egg laid

* Since there exists the possibility that the last egg was held in the reproductive tract for an abnormally long period, it was assumed for this experiment that this egg should have been laid on the eighth day.

** "Per Cent Fertile While Fertile" refers to the percentage of fertile eggs relative to the total number of eggs laid during the duration of fertility.

TABLE 9

Per Cent Successful Massages Performed on Male Kestrels

Year	No. Tiercels	Total No. Trials*	Per Cent Successful Collections	Mean Volume of Semen Obtained Per Successful Trial (ml.)
1974	8	124	77.4	not measured
1975	18	376	71.0	.019
	Total 26	500	Mean 74.2	

* The figures in this column only include those trials attempted on each male after their first successful massages.

TABLE 10

Per Cent Collections Yielding Semen, Urates, Blood and Nil in 1975

Type of Yield	March 12-31	April 1-30	May 1-31	June 1-30	Mean
Semen (S)	24.8 (36)	66.2 (88)	71.4 (105)	54.9 (45)	54.0 (274)
Urates (U)	49.7 (72)	23.3 (31)	15.6 (23)	22.0 (18)	28.4 (144)
Blood (B)	.7 (1)	-	-	-	.2 (1)
SU	4.1 (6)	6.8 (9)	6.8 (10)	1.2 (1)	5.1 (26)
BU	9.7 (14)	-	-	-	2.8 (14)
SUB	1.4 (2)	-	-	-	.4 (2)
SB	.0 (1)	.8 (1)	-	-	.4 (2)
Nil	8.3 (12)	3.0 (4)	6.1 (9)	22.0 (18)	8.5 (43)
Total Attempts	(144)	(133)	(147)	(82)	(506)

almost quadrupled the mean of the other three months. Of the total attempts, semen was collected 54 per cent, urates, 28.4 per cent and nil which ranked third, 8.5 per cent.

The age, weight and semen characteristics of the 18 AI males are summarized in Table 11. Semen volume averaged 13.8 μl, ranging from .1 to 59 μl. The concentration of spermatozoa ranged from 4,000 to 100,000 with a mean of 32,000. The highest sperm count per ejaculate was 3,879,000 while the lowest was only 200 with a mean of 478,000. Motility and semen appearance scores ranged from zero to five with means of 3.9 and 2.5, respectively. The lowest per cent contamination observed was 10 while the highest was 100 with a mean of 68. A noticeable number of abnormal spermatozoa in the first few collections was recorded for four yearlings and two two-year old males.

Referring to Appendix Tables 6A and 6B, significant ($P<0.05$) positive correlations were found between the age of the males and either semen volume or sperm count per ejaculate. With respect to weight of the males, significant ($P<0.05$) and highly significant ($P<0.01$) positive relationships were present with concentration of spermatozoa and sperm count per ejaculate, respectively. Mention is also made of a highly significant ($P<0.01$) positive correlation between age and weight of the males.

The period of semen production, at least in 1975, lasted a total of 103 days (Table 12). The mean duration of semen production among the AI males was 73.6 days, ranging from 48 to 101 days. The mean duration of semen production

TABLE 11

Age, Weight and Semen Characteristics of Individual AI Male Kestrels in 1975

Male No.	Age (Yrs)	Body Wt. (gm)		Per Cent Successful Massages	Semen Volume (μl)		Concentration of Spermatozoa (No. x10^3/mm^3)		Sperm Count per ejaculate (x10^3)	
		Begin	End		Mean	Range	Mean	Range	Mean	Range
57	1	100.8	109.8	85 (23/27)	20	4-44	51	12-100	1013	90.0-2536
27	1	96.4	100.7	73 (11/15)	4	1-10	45	20-70	190	1.9- 674
37	1	100.1	103.2	73 (19/26)	10	2-25	18	7-60	173	.4- 603
38	1	95.1	107.0	77 (10/13)	11	3-43	22	10-40	246	.6- 860
30	1	86.4	107.2	71 (15/21)	9	2-20	38	9-60	287	71.0- 706
32	1	97.2	*100.4	67 (14/21)	7	1-20	34	10-95	225	.6- 796
23	1	94.6	95.9	69 (9/13)	18	2-26	8	6-15	78	.2- 183
56	1	88.2	102.7	58 (14/24)	16	2-20	35	14-60	408	.6- 852
17	1	92.7	97.2	68 (17/25)	15	1-46	17	4-29	240	1.0- 680
784	3	107.1	116.0	72 (18/25)	17	2-33	33	10-60	651	116.0-1962
788	3	131.0	111.4	69 (22/32)	15	1-34	40	4-60	424	5.0-1242
795	3	109.7	122.4	47 (8/17)	29	2-32	36	22-46	573	65.0-1420
787	3	131.5	113.9	59 (13/22)	16	1-59	41	6-60	1876	13.0-3236
773	2	87.1	100.5	71 (17/24)	11	3-34	16	4-36	142	2.5- 318
774	2	114.1	111.1	79 (22/28)	13	2-33	53	6-100	789	.2-2616
776	2	117.1	113.9	91 (21/23)	16	2-42	35	5-54	504	41.0-2082
777	2	118.7	104.1	69 (9/13)	8	2-17	28	10-60	298	28.0- 664
779*	2	109.3	--	71 (5/7)	23	13-43	57	16-94	1456	249.0-3879
Overall Mean		105.6		70.5	13.8	2-32	32	9-59	478	25.7-1261

* bird died midway through season, so it is excluded from averages

TABLE 11 (cont.)

Age, Weight and Semen Characteristics of Individual AI Male Kestrels in 1975

Male No.	Motility Score of Spermatozoa		Semen Appearance Score		Per Cent Contamination	
	Mean	Range	Mean	Range	Mean	Range
57	4.6	2-5	2.7	0-5	86*	50-100
27	3.3	3-5	2.6	0-4	100*	--
37	3.0	0-5	2.6	0-4	83	25-100
38	3.9	1-5	2.3	1-4	49	15-100
30	4.8	3-5	2.1	1-4	66	10-100
32	3.7	5-5	4.0	3-5	92*	65-100
23	2.2	1-5	2.6	0-4	78*	50-100
56	4.9	0-5	1.5	0-3	37	10- 70
17	3.3	1-5	2.3	0-4	60	20-100
784	4.1	2-5	3.6	2-5	74	15-100
788	4.3	2-5	3.6	1-5	55	10-100
795	4.5	3-5	1.4	0-3	97	90-100
787	4.3	1-5	1.7	0-3	43	10- 80
773	3.8	1-5	1.6	0-3	51*	20- 80
774	3.8	0-5	2.6	0-5	68*	10-100
776	4.5	0-5	1.8	1-3	34	10- 50
777	3.8	3-5	2.6	1-4	91	80-100
779	4.7	3-5	2.2	3-5	--	--
Mean	3.9	2-7	2.5	.6-4.5	68	31- 93

* noticeable number of abnormal spermatozoa in first collections

TABLE 12

Duration of Semen Production in Male Kestrels in 1975

Date of first motile sperm	March 19
Date of first measurable sample	March 19
Date of first sample containing 100000 sperm	March 27
Date of last motile sperm	June 30
Date of last measurable sample	June 30
Date of last sample containing 100000 sperm	June 30

for the yearlings was 75.4, while it was slightly higher for the older males, being 82.8 days. In both 1974 and 1975 the same bird produced motile sperm on March 19. In 1975 the last motile sperm were observed on June 30. As seen in Table 13, peak production of semen with respect to semen volume, concentration of spermatozoa, sperm count per ejaculate and motility of spermatozoa occurred between April 7 and 29. The values of these characteristics tapered off from this period to nil on July 10.

In terms of photoperiodic effects, semen production began when the daily light period reached 12 hours and 15 minutes. Peak production was observed between 13 and 14 hours of daily light. This production declined considerably around the longest day of the year, June 21 with approximately 15 hours and 45 minutes of daily light.

No significant ($P>0.05$) correlations (Appendix Table 7) were observed between weather factors, i.e. temperature, barometric pressure and per cent possible daily sunshine, and any of the four aforementioned semen characteristics, as well as per cent successful massages.

The semen characteristics of AI, colony and paired males during the period May 27 to July 10 are presented in Table 14. The colonial males were by far the lowest in all traits with the exception of a high motility due to the examination of two samples only. The AI and paired males were extremely comparable in all traits except for sperm count per ejaculate, where that of the AI males almost doubled the sperm count of the paired males. In each group

TABLE 13

Relation of Semen Characteristics to Time of Year and Photoperiod in 1975

Date	Duration of Daily Light	No. Males Producing Semen	Mean Semen Volume (μl)	Mean Sperm Conc. ($x10^3/mm^3$)	Mean Sperm Count Per Ejaculate ($x10^3$)	Mean Motility Score
Mar. 14	11:50	0/18	--	--	--	--
Mar. 21	12:15	4/18	7	--	8.4	2.8
Mar. 29	12:40	10/18	7	24.5	109.1	3.1
Apr. 7-8	13:05	10/18	19	39.5	662.5	4.5
Apr. 15-16	13:35	13/18	17	61.8	1150.3	4.7
Apr. 21-22	13:50	15/18	13	50.1	579.9	4.5
Apr. 28-29	14:15	14/18	19	34.6	787.3	4.0
May 5-7	14:35	12/17	10	26.3	279.5	4.1
May 19-20	15:05	18/17	12	32.4	214.7	4.3
May 25-27	15:20	13/17	13	27.2	338.0	3.8
June 2	15:30	13/17	12	27.7	632.1	3.7
June 9	15:40	13/17	9	27.0	277.8	3.0
June 16	15:45	9/17	5	43.2	188.5	2.8
June 23	15:45	5/17	2	30.8	166.6	2.7
June 30	15:45	4/17	3	32.7	116.4	1.4
July 10	15:30	0/17	--	--	--	--

TABLE 14

Semen Characteristics of AI, Colony and Paired Male Kestrels During the Period May 27 - July 10

Male Group	Per Cent Successful Massages	Mean Semen Volume (μl)	Mean Sperm Conc. ($x10^3/mm^3$)	Mean Sperm Count ($x10^3$)	Mean Motility Score	Mean Semen Appearance Score	Mean Per Cent Contamination
AI	55 (57/104)	10.0	31.4	384.9	3.1	2.3	70.0
Paired	44 (30/68)	8.3	31.5	196.9	2.8	2.8	78.8
Colony	7 (2/30)	3.7	28.0	106.2	5.0	1.0	30.0

of males, the per cent collections resulting in urates almost equalled that resulting in nil (Table 15). The colonial males were again by far the highest in both percentages with the paired males ranking a solid second in production of urates.

With respect to frequency of collection, semen collections performed on two consecutive days resulted on the second day in a decrease in semen volume 6 out of 7 times and in decreases in sperm concentration and sperm count per ejaculate 4 out of 4 times. A second collection on the same day resulted in reductions of semen volume and sperm concentration 6 out of 9 times and a decrease in sperm count per ejaculate 7 out of 9 times.

In Table 16, the semen characteristics of the American Kestrel are compared with those of several other avian species. The kestrel, the smallest of the birds listed, ranked lowest in concentration of spermatozoa and total sperm count per ejaculate volume but exceeded the quail both in semen volume and duration of fertility.

5.4. Relationship of Semen Characteristics With Fertility and Hatchability

With respect to fertility, the only significant ($P<0.05$) relationship revealed by the Spearman Rank Correlation test (Appendix Table 8) was a positive one with sperm count per ejaculate. However, positive correlations between fertility and both semen volume and concentration of spermatozoa approached significance ($P = 0.05-0.10$).

None of the six semen characteristics was related

TABLE 15

Per Cent Collections Yielding Urates and Nil in AI, Colony and Paired Male Kestrels During the Period May 27-July 10

Male Group	Per Cent Collections Resulting in Urates	Per Cent Collections Resulting in Nil
AI	23.1 (24/104)	22.1 (23/104)
Paired	32.4 (22/68)	23.5 (16/68)
Colony	50.0 (15/30)	43.3 (13/30)

TABLE 16

Comparison of Semen Characteristics Between the American Kestrel and Domestic Avian Species

Species	Approximate Mean Body wt. (Kg)	Mean Semen Vol. (ml)	Mean Sperm Conc. (10^6/cm)	Mean Sperm Count ($x10^6$)	Duration of Fertility (days)
Turkey	11.4[a]	200-300[c]	6 - 8[c]	1750[h]	40 - 50[c]
Goose	7.2[a]	50-600[c]	-----	----	9.7[e]
Duck (large)	4.5[a]	450[d]	1.57[d]	624.9[d]	8[f]
Pigeon	.8[a]	10-20[b]	.37[h]	5 - 6[b]	8[b]
Chicken (large)	4.1[c]	500-800[c]	3.5[c]	1750[c]	10 - 13[c]
Quail	.2[a]	10[c]	5 - 6[c]	55[h]	4 - 5[c]
Kestrel	.1	14	.034	.53	8.1

a adapted from Jull (1947)
b adapted from Owen (1941)
c adapted from Smyth (1968)
d adapted from Pingel (1972)
e adapted from Johnson (1954)
f adapted from Watanabe and Sugimori (1957)
g adapted from Ensminger (1971)
h author's calculations

significantly ($P>0.05$) with hatchability.

Significant relationships among semen characteristics are shown in Appendix Tables 9A, 9B and 9C. Per cent successful massages and semen volume exhibited a significant ($P<0.05$) negative relationship. There also exists a highly significant positive ($P<0.01$) correlation between sperm count per ejaculate and concentration of spermatozoa. Furthermore, the Spearman Rank Correlation test showed highly significant ($P<0.01$) and significant ($P<0.05$) relationships between motility of spermatozoa and either concentration of spermatozoa and sperm count per ejaculate, respectively.

Attempts to relate duration of motility under storage conditions and either fertility or hatchability were not made, but some data were collected on pooled samples to determine effective storage conditions of kestrel semen. The mean motility of three samples of pure semen held under refrigeration, i.e. 4.4 to 10°C, dropped from 5 to 3.5 after 12 hours and then to 2.3 after 24 hours. Barely motile sperm were still observed at 96 hours. At room temperature, the mean motility of three similar samples dropped from 5 to 2.7 after 12 hours and slight motility was visible up to 72 hours. The mean motility of sperm in three semen samples mixed equally with Wilcox phosphate buffer declined after 12 hours from 5 to 3.7 under both room and refrigerated temperatures. After 24 hours it decreased to 3.5 in two samples held at room temperature and to 3 in three samples stored in the refrigerator. In samples mixed with Wilcox phosphate buffer, barely visible motility was seen up to 72 hours in one held

at room temperature and up to 84 hours in another kept in the refrigerator.

6. DISCUSSION

6.1. Egg Production, Fertility and Hatchability

In general, single AI females were more inclined to lay in exposed conditions while paired females laid more frequently in isolated quarters. This agrees with the suggestions of Owen (1941) that induction of laying in female pigeons that do not ordinarily lay in isolation may be achieved by placing males and females in adjacent cages or by placing two females in the same cage. However, two attempts with the latter technique using two female kestrels and two nestboxes in 1975 resulted in eggs broken on the ground on both occasions. In contrast, all three females held colonially with seven males in a large pen laid eggs in three different nestboxes in 1975.

Although fertility in the NM females was not greatly affected by pen design, the per cent hatchability was much lower in both artificially and naturally incubated eggs of NM females held in exposed quarters. The reasons for this low hatchability will be discussed in the next section.

The overall per cent fertility obtained with AI as compared to that obtained by NM is excellent proof of the success of the technique in producing fertile eggs. Experience with the technique may have played some part in the increases of fertility from 1974 to 1975.

One disadvantage of the AI technique might be the

failure to fertilize those eggs laid or too developed prior to insemination. This may be insignificant however, in consideration of the almost equal numbers of fertile eggs obtained by either breeding technique. Although goshawks were successfully inseminated prior to egg-laying (Berry, 1972; Corten, 1973), this was not possible with the American Kestrel.

Natural incubation by single AI females was generally not practiced. In 1974 second clutches were left to be incubated by the four AI females who laid them. All clutches including two containing fertile eggs were abandoned by the females, so the eggs were placed in an incubator. Although in 1975 one AI female successfully hatched a chick out, the overall tendency to lose interest in incubation may result from the absence of a male to relieve the incubating female. On many occasions, NM males were observed relieving their incubating mates and at least twice were seen sitting beside their mates on the eggs.

The failure to find significant differences in hatchability of eggs produced by either breeding technique and incubated either naturally or artifically provides further evidence of the success of AI. Although the hatchability dropped for all treatments from 1974 to 1975, most disturbing was the more than 50 per cent drop in hatchability of eggs laid by AI females and artificially incubated. This has been reported as early as 1938 when Burrows and Marsden (1938) found a significantly higher embryonic mortality in eggs from AI turkey hens than in eggs from NM females,

but offered no explanation as to the exact cause.

The following possibilities are suggested as causes of the poor hatching results obtained in 1975. Unseasonably high temperatures in the month of May could account for the poor hatchability in all treatments, i.e. NM vs AI, artificial vs natural incubation. This month now holds the records in the Montreal area at least for the highest mean maximum temperature, the second highest mean minimum temperature and the highest monthly mean temperature, the latter being 17.7°C as compared to only 10.4°C in May 1974. Prior to May 27, 22 out of 24 fertile eggs in 1975 hatched successfully for a per cent hatchability of 91.7 which compares well with the 95 per cent overall hatch obtained in 1974 in the same incubator. Out of 65 fertile eggs artificially incubated due to hatch after May 27 in 1975, only 23 hatched for a per cent hatchability of 35.4. Furthermore, only 12 out of 30 naturally incubated fertile eggs also due to hatch after May 27 hatched for a per cent hatchability of 40. Embryos died at various stages of development including 10 fully-developed chicks which died at the pipping stage. In poultry breeding, it is known that storage of eggs in warm temperatures prior to incubation impairs hatchability (Landauer, 1951).

The slight differences in the design of the Marsh Farms Roll-X incubator bought in 1975, i.e. thinner walls, as compared to the 1974 model may bear some importance, as the former seemed to possess an inherent higher relative humidity even though volume and surface area of the water were virtually the same in both incubators. Several laboratory

thermometers were used to test this, as the thermometers sold with the incubators are of lesser quality and hence less accurate. On May 27, 18 out of 26 fertile eggs kept in the 1975 incubator held dead embryos. The remaining eight were moved to the 1974 incubator which had already hatched 22 out of 24 eggs, but only one of these hatched. The total per cent hatchability for the 1974 incubator in the years 1974 and 1975 were 95 and 69.5, respectively.

The third possibility is the effect of the AI method of mating on the hatchability. Of the 26 fertile eggs held in the 1975 incubator prior to May 27, 15 were produced by AI. The single egg that eventually hatched in the 1974 incubator was an AI egg. Included in the 22 of 24 eggs successfully hatched in the 1974 incubator prior to May 27 were three AI eggs. Finally, 22 out of 39 fertile AI eggs kept in the 1974 incubator successfully hatched after May 27. In view of the excellent hatch of AI eggs obtained in 1974 by artificial incubation (18 out of 19), it seems doubtful that AI eggs are inferior to NM eggs in hatchability.

The influence of pen design has already been mentioned. The low hatchability of NM eggs both artificially and naturally incubated laid under exposed conditions in both 1974 and 1975 certainly makes further research into this aspect extremely important.

With respect to bacterial contamination reported in eggs laid by captive kestrels (Porter and Wiemeyer, 1970), it is difficult to draw any conclusions as to whether this is a cause or a consequence of embryo mortality. Although environmental cleanliness in pens and the incubator room was equally

maintained in either year, this factor cannot be entirely ruled out. It is not impossible that contaminated eggs moved from the unsuccessful 1975 incubator on May 27 to the 1974 incubator may have resulted in further contamination and a poorer hatch in the latter incubator.

Increasing age of parents was also proposed by Porter and Wiemeyer (1970) as a possible cause for the poor hatch of eggs laid by non-yearling pairs in 1968. This might not pertain to my experiments, as yearlings were paired with older birds.

In retrospect however, it seems plausible that a combination of both unseasonably high temperatures in the month of May where the greatest hatching occurs and the inherent qualities of the 1975 incubator were most likely responsible for the poor hatching results in 1975.

The high fledging success of hand-raised birds as compared to parent-raised birds is a strong indication of an excellent procedure for hand-raising kestrel chicks, thereby enhancing the value of the American Kestrel as a research animal. Also, there is no doubt in the author's mind that kestrels produced by AI are as healthy as those bred naturally. In fact, some of the AI birds produced in 1974 successfully mated and fledged young in 1975, probably constituting the first birds of prey in history ever to do so.

6.2. Duration of Fertility

Since fertile eggs appeared only two days after AI 41.2 per cent of the time, this indicates an onset

of fertility of about one day, allowing for one day of egg development. If 'capacitation' of spermatozoa, a phenomenon described for mammalian sperm (Chang, 1951), but also suggested for quail (Ogasawara and Huang, 1963) does occur in the kestrel, it will require at most one day. The duration of fertility of 8.1 days indicates the presence of sperm storage glands in the American Kestrel as reported in chickens (Bobr et al, 1962), turkeys (Verma and Cherms, 1964), and both pheasants and quail (J. J. Makos III in Smyth, 1968). Further research on other species of birds of prey would probably reveal as much, although Corten (1973) suggests that sperm storage glands do not exist in goshawks because of observations of numerous copulations required to fertilize one clutch in the wild (Holstein, 1941 in Corten, 1973). On one occasion, a fertile egg was produced at least nine days after the previous egg was laid, giving a supposed duration of fertility of 15 days. This is not conclusive however, as there is the possibility that the fertilized egg was held in the system for the unusually long period between ovipositions.

In view of the above duration of fertility, the author recommends twice weekly inseminations, but to ensure the highest fertility, one cannot go wrong by inseminating after every oviposition.

6.3. Semen Production

6.3.1. Per Cent Successful Massages

The little difference in per cent successful massages from year to year indicates that experience with the

technique of collection does not play a large part in the successful procurement of semen. In 1974 the role of the operator who collects the semen in the tube was comprised of many different individuals, some with no experience. In 1975 this role was undertaken by one person. The importance of experience is mainly stressed for the collection of good quality semen free of urates, as already demonstrated in chickens (Burrows and Quinn, 1938).

The high percentage of collections resulting in urates in the month of March was likely due to the feeding of males prior to collections on collection days. This percentage was decreased by more than half by simply not feeding the AI males until all collections were performed on that day. The 11.8 per cent collections resulting in blood-tinged samples occurred almost solely in March. The situation was remedied by placing pressure on the abdominal area just below the cloaca with the thumb of the hand holding the bird's legs during milking of the semen. Less pressure by the milking fingers is needed and consequently, the chances of haemorrhaging greatly decreased. Extremely cold temperatures and collections made in the beginning of the 1975 season perhaps with more zeal and less care than in 1974 may have also contributed to the cause.

On four occasions, semen was ejaculated with little or no massage required, indicating that some of the males were being conditioned to the technique. It is commonly known in poultry that males will respond to the massage technique exceptionally quickly after a period of practice.

In two of these instances, the semen examined was contaminated with as much and more debris than samples collected previously by full massage in the same bird. This is not in agreement with the finding of Kamar (1958) that cock semen obtained by collection without milking is relatively free of contaminants compared to that milked from the bird.

6.3.2. Factors Influencing Semen Production

6.3.2.1. Influence of Age

The significant positive correlations found between age of the males and either semen volume or sperm count per ejaculate volume does not agree with the results obtained in fowl (Penquite et al, 1930; Burrows and Titus, 1939) or those reported in turkeys (Carson et al, 1955a). They were likely due to the little variability in these characteristics expressed by the older birds. However, some yearlings were extremely capable of producing volumes of semen and sperm numbers that equalled or surpassed those of some of the older birds. Increasing age did not influence the duration of semen production to any large extent as suggested by the experiments of Corten (1974) when his tiercel goshawk produced semen later in the season than it had previous years.

High percentages of abnormal spermatozoa in young cockerels have been reported by Sampson and Warren (1939) and Van Drimmelin (1951). In the American Kestrel, noticeable numbers of abnormal spermatozoa were observed only in the early collections of four yearlings and two older birds.

6.3.2.2. Effect of Body Weight

This study revealed no positive correlation between

body weight of the males and ejaculate volume as reported in fowl by Penquite et al (1930) and Hartmann and Gleichauf (1974). Also contrary to the observations of Penquite et al (1930) concerning a negative correlation, a significant positive relationship was shown between body weight and concentration of spermatozoa.

6.3.2.3. Individual Variability

There appears to be a reasonable amount of individual variability with regard to all semen characteristics in the kestrel. This agrees with the finding of Carson et al (1955b) of considerable variation in semen volume in different male turkeys. It only agrees partially with McCartney (1956) who found no significant differences among toms for semem volume, motility, pH or sperm concentration, but did for sperm numbers per ejaculate volume.

6.3.2.4. Time of Year

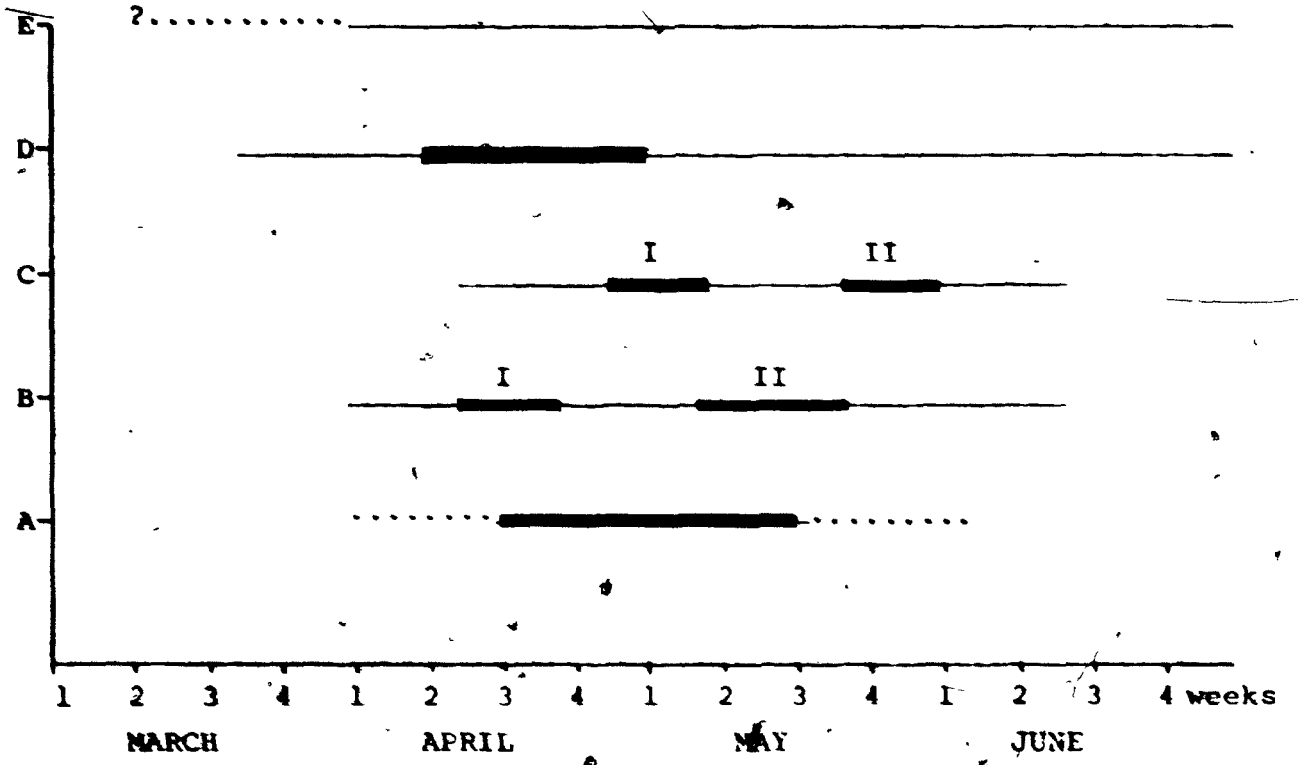
Since semen production did not begin both years until at least March 19 and continued up to June 30 in 1975, a definite seasonal production is confirmed in the kestrel as already demonstrated in chickens (Parker and McSpadden, 1943), turkeys (Carson et al, 1955a) and starlings (Bissonnette 1930 in Bissonnette and Wadlund, 1932).

With respect to duration of semen production, the mean of 73.6 days ranging from 48 to 101 days reported in this work agrees reasonably well with the 111 and 70 day periods observed in two Golden Eagles (Grier, 1973) as well as that shown in goshawks (Corten, 1973). The somewhat shorter periods of attempted copulations in hawks described by Berry

(1972) and Bird and Laguë (in press) do not necessarily signify periods of semen production, as motile sperm were collected from paired kestrel males long after egg-laying ceased. Although no dates of commencement were recorded for paired males, the termination dates of semen production in both AI and paired males were very close.

A definite peak in semen production during the last three weeks of April coincides extremely well with that reported in chickens (Parker and McSpadden, 1943) and with testes development in starlings (Bissonnette and Chapnick, 1930). It also falls into the period of maximum sperm production for turkeys (Carson et al, 1955a).

Berry (1972) stated that semen production was closely synchronized with egg-laying in his goshawks. Figure 12 summarizes the approximate laying times of wild and captive kestrels and the periods of semen production of both AI and NM males. Egg-laying in the paired NM females usually took place during the last two weeks of April while the single AI females with few exceptions laid eggs one to two weeks later than the NM females. The egg-laying times of the NM birds coincided quite closely with those of wild kestrels laying eggs in the Montreal area in 1973 and 1974 (Bird and Spiegel, 1975). It is clearly evident that semen production of the AI males is closely coincident with the egg-laying periods of both wild and captive pairs of kestrels in the Montreal area. It is also apparent that some underlying factor common to all of the above birds is responsible for this close synchronization of semen production and egg-



A - Beginning of first clutches in wild kestrels nesting in Montreal in 1973 and 1974
B - Beginning of first (I) and second (II) clutches in captive paired females in Montreal
C - Beginning of first (I) and second (II) clutches in captive AI females in Montreal
D - Semen production of AI males isolated from females
E - Semen production of paired NM males

Figure 12. Approximate Periods of Egg-laying and Semen Production of Wild and Captive Kestrels

laying.

6.3.2.5. Influence of Photoperiod.

That photoperiod or the lengthening daily sunlight period is probably the most important factor influencing gonadal cycles in birds has already been shown by Bissonnette (1936), Rowan (1938), Farner (1959), Wolfson (1959) and Schwab (1970). Different lighting regimes have been reported to affect both semen production and semen quality in chickens (Lamoreux, 1943; Bonadonna and Pozzi, 1955; Bajpai, 1963; Parker and McCluskey, 1964). The importance of photoperiod as a stimulatory factor in egg-laying in American Kestrels has already been shown by Willoughby and Cade (1964). Since peak production of semen was closely synchronized with egg-laying in this study, one can conclude that photoperiod plays a significant role in stimulating semen production in the American Kestrel. It is not yet known whether semen production in American Kestrels could be prolonged for extended periods by holding the photoperiodic regime at peak production as was done in starlings in California (Schwab, 1970).

The laying of eggs by the single AI females one to two weeks later than NM females is a problem that must be solved. Attempts to fertilize a second clutch of eggs of the AI females in 1974 by removing first clutches were for the most part foiled because the semen production of the AI males was declining rapidly at the time of that laying. A possible solution might be the alteration of photoperiodic regimes of either the females or the males so that semen production of the AI males and egg-laying of the AI females

are more closely synchronized, i.e. lag the photoperiod of the AI males two weeks behind or advance the photoperiod of the AI females by two weeks.

6.3.2.6. Influence of Weather

In concordance with Bissonnette's (1931) conclusions that temperature and barometric pressure are not regulatory factors in sexual changes in starlings, these variables as well as per cent possible sunshine were found not to affect semen production or semen quality. In fact, good quality semen was collected at temperatures below -15°C and above 30°C. This is in complete agreement with results reported for fowl (Wheeler and Andrews, 1943). It has already been shown that kestrels can produce fertile eggs in winter regardless of temperature (Willoughby and Cade, 1964).

6.3.2.7. Management of Males

The results of Table 14 seem to indicate that males kept in small quarters isolated from females are superior to both paired males and colonial males in almost every semen characteristic including per cent successful massages. This is not congruent with the work of Lorenz et al (1956) who found no significant differences in semen volume or sperm concentrations between paired and non-mated turkey toms. The results reported here are not necessarily conclusive however, as the AI males were handled regularly prior to May 27, whereas the paired and colonial males were not. In support of the results are the observations of Burrows and Quinn (1937) in fowl and Owen (1941) in pigeons that males kept in large pens or males paired with females may be

ejaculated regularly but give smaller yields of semen.

There are two advantages to keeping males in small quarters either tethered in falconer's fashion or held in box cages. Firstly feeding is more easily controlled and thus one can reduce the per cent collections contaminated with urates. In this regard the AI males held in box cages had lower percentages than either paired or colonial males. Secondly, the stress and trauma associated with catching males in large pens is considerably reduced by keeping males in quarters where they can be readily and easily caught. Although Rowan (1928) in his early work on juncos suggested that exercise promotes gonadal development in birds, Bissonnette (1931) observed no such effect in his starlings with additional exercise. In this study, restricted space, hence possible lack of exercise of the AI males had no negative influence on their semen production.

6.3.2.8. Nutritional Effects

Since semen collected in 1974 was rarely examined microscopically, it is virtually impossible to compare semen quality between 1974 and 1975 when the food of the AI males was supplemented by additions of calcium and phosphorus in the form of bone meal. Although the per cent successful massages was higher in 1974, the fertility was considerably lower in 1974. Ganders when fed a high calcium diet gave increased sperm concentration and viability (Molnar et al, 1971; Kovacs, 1972), and when fed a high phosphorus diet, gave increased sperm concentration and ejaculate volume (Kovacs, 1972). From these results, it is possible that

the increased dietary calcium and phosphorus of AI kestrel males may have been responsible for the greatly increased fertility. Further research is required however, before any solid conclusions can be drawn.

6.3.2.9. Effects of Frequency of Collection

The few observations on the effects of frequency of collection did indicate that both daily and twice daily collections reduced the semen volume, concentration of spermatozoa, and sperm numbers per ejaculate volume. This finding is in complete agreement with those reported for fowl (Ponquite et al, 1930; Sampson and Warren, 1939), for turkeys (Lorenz et al, 1956; McCartney et al, 1958; Nestor and Brown, 1971), but only partially for hawks (Temple in Grier et al, 1972) as daily collections in goshawks did not appear detrimental to semen quality (Corten, 1973). The decrease in sperm numbers in twice daily collections also lends support to Owen's (1941) feelings that regular collections more than once a day causes male pigeons to become aspermic. To obtain good semen quality in kestrels minimum and maximum intervals between collections appear to be respectively two days and roughly one week. The same recommendations exist for chickens (Smyth, 1968) and turkeys (Lorenz et al, 1956).

6.3.3. Relationship of Semen Characteristics with Fertility and Hatchability

The absence of any relationship between motility and fertility agrees with the findings of McCartney (1956), Grosse 1957 in McDaniel and Craig (1962) and Boone and Huston (1963).

However, as most semen samples used for AI in kestrels have a high motility score, it is possible that the little variability in this factor may have masked a true correlation. In fowl a positive correlation has been shown by many researchers (Shaffner and Andrews, 1948; Allen and Champion, 1955; Cooper and Rowell, 1958; McDaniel and Craig, 1959, 1962; Kamar, 1960; Smyth, 1968; Kammerer et al, 1972; Mymrin et al, 1972; Kamar and Razik, 1973). I think that motility is a valuable indicator of semen quality.

The results pertaining to duration of motility under storage conditions compare with that reported for eagles (Grier, 1973), goshawks (Corten, 1973) and fowl (Garron and Shaffner, 1952; Hunsaker et al, 1952). Sperm motility up to 12 hours was better maintained at 4 to 10°C and the longest duration was also observed here. At room temperature, the duration of motility in samples mixed with Wilcox phosphate buffer was only slightly shorter and compared well with similar samples held at refrigerated temperatures. The main disadvantage of holding samples at room temperature was bacterial growth which was never seen in refrigerated samples. The overall results seem to indicate that motility of kestrel sperm is best maintained for at least up to 12 hours when mixed with an equal amount of Wilcox phosphate buffer and kept in the cold.

Semen volume was not significantly related to fertility. In fowl, only two reports of such a correlation exist (Kamar, 1960; Kamar and Razik, 1973), while the majority of researchers (Shaffner and Andrews, 1948; McCartney, 1956; Boone and

Huston, 1963; Smyth, 1968; Hartmann and Gleichauf, 1974) found no evidence of this relationship.

Similarily, the absence of a significant relationship between concentration of spermatozoa and fertility is congruent with the findings of Shaffner and Andrews (1948), McCartney (1956) and Boone and Huston (1963) in fowl, although several other fowl researchers, some more recent, believe it to exist (Cooper and Rowell, 1958; Kamar, 1960; Mymrin et al, 1972; Kamar and Razik, 1973; Hartmann and Gleichauf, 1974).

The colour of kestrel semen varies from clear to deep amber. The amber colour was examined microscopically and was not found to result from contamination by blood. This colour has not been observed previously either in hawk semen (Grier et al, 1972; Corten, 1973) or in semen of large falcons (Fyfe, Enderson, personal communications). Although semen colour was not significantly correlated with any other semen characteristics or fertility or hatchability, it did appear that the more amber the samples were, the more highly contaminated they were with epithelial debris. Decreased motility of the spermatozoa was observed in deep amber samples. Viscous, opaque samples usually were heavily contaminated with urates. Therefore, one might evaluate semen quality by its colour which supports the claim by Brown and Graham (1971) in turkeys.

The only characteristic, sperm count per ejaculate, to be significantly and positively correlated with fertility was not found to have such a relationship in fowl (Sampson

and Warren, 1939; Hartmann and Gleichauf, 1974).

The contaminants described in goshawk semen by Corten (1973) were only occasionally observed in kestrel semen. Most debris consisted of epithelial cells. Since inseminations were performed almost immediately after collections, fecal contamination likely did not affect fertility as reported for fowl by Smyth (1968).

Noticeable numbers of abnormal spermatozoa were present only in samples collected early in the season. Normal spermatozoan heads during locomotion had a somewhat elongated jelly-bean shape (Fig. 13). One head measured roughly four microns long and two microns wide and the tail length was approximately 15 to 20 microns. The overall description differs in both appearance and size with that for spermatozoan heads of large falcons (Corten, 1973, 1974). Locomotion consisted of a counterclockwise turning of the entire body. They were capable of moving at a mean speed of 2.6 mm/min., ranging from 2.4 to 3. Major abnormalities observed were elongated heads; blunted heads, tiny heads which may belong to immature sperm, and extreme clumping of sperm around debris. All of these have been reported in poultry (Sampson and Warren, 1939; Bajpai, 1963). Further research in the area of raptor sperm morphology should be encouraged as several researchers have been able to differentiate between varieties of fowl by variations in the spermatozoan heads (Kashiwabara, 1964; Sharma and Sidha, 1971; Sinha, 1971). This may prove a valuable taxonomic tool.

Figure 13. Microscopic (700X, phase contrast, green filter) view of spermatozoa collected from an AI male kestrel. Note the somewhat elongated jelly-bean shape of the head and the dense epithelial debris surrounding the sperm.

None of the correlations described in fowl between semen volume and motility (McDaniel and Craig, 1959; Mymrin et al, 1972), or semen volume and concentration of spermatozoa (Carson et al, 1955b) were recorded in this study of the American Kestrel. The positive relationship between motility and concentration of spermatozoa reported here is however, in agreement with the findings of McDaniel and Craig (1962) and Mymrin et al (1972), but is not consistent with the negative relationship reported by Nishiyama et al (1971). Both correlations between sperm numbers per ejaculate volume and either motility or concentration have not been recorded before in any avian species.

The significant negative relationship between per cent successful massages and semen volume seems to indicate that the greater the number of successful massages made on the male, the smaller will be the mean volume of semen of that particular male. The inclusion of the few collections made daily and twice daily into this analysis and the reduction of the semen volume produced as the season progresses may have affected this relationship.

In turkeys, Kamar (1960) and Kamar and Razik (1973) have defined significant positive relationships between hatchability and either motility or concentration of spermatozoa, but not semen volume. This study revealed no correlations between hatchability and each of the six semen characteristics.

Parker's work on turkey semen production in 1946 suggests a negative relationship between semen volume and

sperm concentration. Turkeys produce less than half the semen volume produced by chickens, but usually have twice the concentration of spermatozoa, which roughly equalizes the number of sperm per ejaculate. The results obtained on the kestrel appear to contradict this suggestion. Furthermore, a relation between size of a species and its mean concentration of spermatozoa does not seem likely, as the tiny quail produces far more sperm per ejaculate than the equally small kestrel. Likewise, the duration of fertility of the kestrel is highly comparable to that of the much larger ducks and geese. With the exception of the 50 μl of semen produced by goshawks, it appears that Golden Eagles and Red-tailed Hawks are capable of producing 10 times as much semen as kestrels in a single ejaculation (Grier et al, 1972).

6.4. Benefits of Artificial Insemination

The following are listed as present and future benefits of the technique of artificial insemination as adapted to birds of prey:

1) A well-proven technique of artificial insemination will allow raptor breeders to make better use of birds not willing to mate naturally.
2) The technique of semen collection and insemination should allow researchers to gain further insight into the reproductive physiology of birds of prey, which is greatly lacking at present.
3) The collection and examination of raptor semen without sacrificing birds may become a useful and important parameter in measuring effects of chemical contaminants in

in the environment on reproductive functions of raptors.

4) The use of artificial insemination may further pave the way for genetically-controlled breeding experimentation, i.e. to determine effects of inbreeding or to produce 'super-hawks' more suitably adapted for a constantly and rapidly changing environment.

5) The microscopic examination of morphological features of spermatozoa collected by an inexpensive, harmless means may prove to be a valuable taxonomic tool in differentiating species and subspecies of raptorial birds.

7. SUMMARY AND CONCLUSIONS

Methods for semen collection and intravaginal insemination for small falcons have been described in detail. Data was collected on both techniques to fully determine their value. The period of semen production in American Kestrels was determined and the factors affecting semen production were examined. Also studied was the duration of fertility in female kestrels. Several semen characteristics were evaluated and related to fertility, hatchability and to each other.

The following conclusions appear warranted from the above studies:

(1) In proportion, more single AI females laid eggs when exposed to other social mates, i.e. mated pairs, single females than when isolated.

(2) In NM pairs, the hatchability of eggs laid in isolated pens was considerably higher than that of those laid in exposed pens, under both artificial and natural incubation.

(3) There were no significant differences between mating types, i.e. AI vs. NM, with respect to per cent laying females producing fertile eggs.

(4) The overall fertilities from either mating type were extremely close (AI = 57% NM = 61%), while the fertility

from AI was almost 15 per cent higher than the fertility of the NM pairs.

(5) No significant differences were present between the hatchabilities of eggs produced by either mating type under artificial and natural incubation.

(6) The onset of fertility was about one day and the mean duration of fertility was determined to be 8.1 days, pointing at an insemination program of about twice weekly.

(7) The massage technique was successful in obtaining measurable volumes of semen 74.2 per cent of the time.

(8) Collecting the semen prior to feeding significantly reduced the per cent collections with urates.

(9) The mean volume of semen per collection was 14 μl; the mean sperm concentration, 32,400 sperms/mm; the mean sperm count per ejaculate, 477,500; the mean motility score, 3.9; the mean per cent contamination, 68; and the mean semen appearance score, 2.5.

(10) The age of males was significantly and positively correlated with both semen volume and sperm count per ejaculate.

(11) The body weight of the males was significantly and positively correlated with both sperm concentration and sperm count per ejaculate.

(12) In 1975 the duration of semen production was 103 days, beginning on March 19. The mean duration of semen production in 1975 among 17 males was 73.6 days and was slightly higher for older males.

(13) Semen production of the AI males appeared to be closely syhchronized with egg-laying of both captive and wild kestrels in the Montreal area.

(14) Semen production began in male American kestrels when the daily natural photoperiod reached 12 hours and 45 minutes. Peak production was observed between 13 and 14 hours of daily light. Production declined considerably around the longest day of the year, June 21, with approximately 15 hours and 45 minutes of daily light.

(15) No significant correlations were detected between any semen characteristics, as well as per cent successful massages and weather factors, i.e. temperature, barometric pressure, per cent possible sunshine.

(16) Males kept in small quarters isolated from females surpassed both paired males and males kept colonially in a large pen with females in semen production and semen quality during the period May 27 to July 10. Restricted movement had no negative effects on semen production and quality.

(17) Daily and twice daily collections proved detrimental to semen volume, sperm concentration and sperm count per ejaculate.

(18) Fertility was significantly and positively correlated with sperm count per ejaculate.

(19) No semen characteristic was significantly related to hatchability.

(20) Among semen characteristics, significant positive correlations existed between sperm count per ejaculate and sperm concentration; motility and sperm concentration; and motility and sperm count per ejaculate. Per cent successful massages and semen volume were significantly and negatively related.

(21) It was possible to maintain reasonably good motility for up to 12 hours when kestrel semen was mixed in equal parts with Wilcox phosphate buffer and stored refrigerated.

(22) Sperm heads were of an elongated jelly-bean shape, roughly four microns long and two microns wide; tail length was 15 to 20 microns; locomotion at a mean speed of 2.6 mm per min consisted of a counterclockwise turning of the entire sperm body. Major abnormalities were elongated heads, blunted heads, tiny heads (immature?) and extreme clumping of sperm around debris.

(23) In comparison with six other avian species previously studied, the American Kestrel, lightest in body weight, ranked lowest in sperm concentration and sperm count per ejaculate, but exceeded the quail of similar but slightly heavier size in both semen volume and duration of fertility.

LITERATURE CITED

Allen, C. J. and L. R. Champion. 1955. Competitive fertilization in the fowl. Poultry Sci. 34: 1332-1342.

Allen, T. E. and G. W. Grigg. 1957. Sperm transport in the fowl. Aust. J. Agr. Res. 8: 788-799.

Bajpai, P. K. 1963. The effect of photoperiodicity on semen characteristics of poultry. Poultry Sci. 42: 462-465.

Berry, R. B. 1972. Reproduction by artificial insemination in captive American goshawks. J. Wildl. Mgmt. 36(4): 1283-1288.

Bird, D. M. and P. C. Laguë. 1975. Successful captive breeding of American rough-legged hawks. Raptor Res., in press.

Bird, D. M. and S. Spiegel. 1975. Unusual food transfer behavior performed by American kestrels. Can. Field-Nat. 89: 325-326.

Bissonnette, T. H. 1931. Studies on the sexual cycle in birds. IV. Experimental modification of the sexual cycle in males of the European starling (Sturnus vulgaris) by changes in the daily period of illumination and of muscular work. J. Exp. Zool. 58: 281-319.

Bissonnette, T. H. 1936. Sexual photoperiodicity. J. Heredity 27: 171-180.

Bissonnette, T. H. and M. H. Chapnick. 1930. Studies on the sexual cycle in birds. II. The normal progressive changes in the testis from November to May in the European starling (Sturnus vulgaris), an introduced, non-migratory bird. Amer. J. Anat. 45: 307-343.

Bissonnette, T. H. and A. P. R. Wadlund. 1932. Duration of testis activity of Sturnus vulgaris in relation to type of illumination. J. Exp. Biol. 9: 339-350.

Bobr, L. W., F. W. Lorenz and F. X. Ogasawara. 1962. The role of the uterovaginal junction in storage of cock spermatozoa. Poultry Sci. 41: 1628 (Abstr.).

Bonadonna, T. 1939. Artificial insemination of birds. Seventh World Poult. Congr. and Exp. Proc. pp. 79-82.

Bonadonna, T. and G. G. Pozzi. 1955. The effect of light on semen production in Gallus gallus. Zootech. e. Vet. 10: 43-61.

Boone, M. A., and B. L. Hughes. 1970. Contamination of semen and its effect on avian fertility. Poultry Sci. 49: 402-404.

Boone, M. A., and T. M. Huston. 1963. Effects of high temperature on semen production and fertility in the domestic fowl. Poultry Sci. 42: 670-676.

Bornstein, S., H. Schindler, I. Gabriel, and E. Moses. 1960. Fertilization rate of chickens inseminated in the morning or in the afternoon. Ktavim 10: 183-191.

Boyd, L. 1973. Personal communication. Moscow, Idaho.

Brown, K. I., and E. F. Graham. 1971. Effect of semen quality on fertility in turkeys. Poultry Sci. 50: 295-296.

Burrows, W. H., and S. J. Marsden. 1938. Artificial breeding of turkeys. Poultry Sci. 17: 408-411.

Burrows, W. H., and J. P. Quinn. 1935. A method for obtaining spermatozoa from the domestic fowl. Poultry Sci. 14: 251-254.

_____. 1937. The collection of spermatozoa from the domestic fowl and turkey. Poultry Sci. 16: 19-24.

_____. 1938. Effective dosages of undiluted semen in artificial insemination of chickens. Poultry Sci. 17: 131-135.

Burrows, W. H., and Harry W. Titus. 1939. Some observations on the semen production of the male fowl. Poultry Sci. 18: 8-10.

Carson, J. D., F. W. Lorenz, and V. S. Admundson. 1955a. Semen production in the turkey male. 1. Seasonal variation. Poultry Sci. 34: 366-343.

_____. 1955b. Semen production in the turkey male. 3. Quantities produced. Poultry Sci. 34: 348-355.

Chang, C. M. 1951. Fertilizing capacity of spermatozoa deposited into the fallopian tubes. Nature 168: 697-698.

Cooper, D. M., and J. G. Rowell. 1958. Relations between fertility, embryonic survival and some semen characteristics in the chicken. Poultry Sci. 37: 699-707.

Corten, P. J. M. 1973. Through method and machination. Captive Breeding of Diurnal Birds of Prey 1(4): 3-5.

_____. 1974. Further experience, progress and success with AI in birds of prey during 1974. Captive Breeding of Diurnal Birds of Prey 1(5): 12-13.

Crew, F. A. E. 1926. On fertility in the domestic fowl. Proc. Roy. Soc. Edin. 46: 230-238.

De Silva, P. L. G. 1963. Evidence for absence of an effect of temperature shock on fertilizing ability of fowl spermatozoa. J. Reprod. Fertil. 6: 371.

Dunn, L. C. 1927. Selective fertilization in fowls. Poultry Sci. 6: 201-214.

Enderson, J. H. 1974. Personal communication. Fort Collins, Colorado.

Enderson, J. H. and D. D. Berger. 1970. Pesticides: egg-shell thinning and lowered production of young in prairie falcons. BioScience 20: 355-356.

Ensminger, M. E. 1971. Poultry science. The Interstate Printers & Publishers, Inc., Danville, Illinois.

Farner, D. S. 1959. Photoperiodic control of annual gonadal cycles in birds. in Withrow, R. B., Photoperiodism and related phenomena in plants and animals. Amer. Assoc. Adv. Sci., Washington, D. C. pp. 717-750.

Fessner, W. 1970. Success breeding peregrine falcons. Captive Breeding of Diurnal Birds of Prey 1(1): 22.

Fyfe, R. W. 1974. Personal communication. Canadian Wildlife Service, Edmonton, Alberta.

Fyfe, R. W., J. Campbell, B. Hayson, and K. Hodson. 1969. Regional population declines and organochlorine insecticides in Canadian prairie falcons. Can. Field-Nat. 83: 191-200.

Garren, H. W. and C. S. Shaffner. 1952. The effect of temperature and time of storage on the fertilizing capacity of undiluted fowl semen. Poultry Sci. 31: 137-145.

Grier, J. W. 1973. Techniques and results of artificial insemination with golden eagles. Raptor Res. 7(1): 1-12.

Grier, J. W., R. B. Berry, and S. A. Temple. 1972. Artificial insemination with imprinted raptors. J. N. Am. Falconers' Assoc. 11: 45-55.

Hartmann, W., and R. Gleichauf. 1974. Effect of line on semen production in white leghorn cocks and its relationship with fertility. Zuchthygiene 9(2): 77-78, cited in A. B. A. 43: 559, 1975.

Hickey, J. J., ed. 1969. Peregrine falcon populations: their biology and decline. University of Wisconsin Press, Madison, U.S.A.

Howarth, B. Jr. 1971. Transport of spermatozoa in the reproductive tract of turkey hens. Poultry Sci. 50: 84-89.

Hughes, B. L., and J. E. Parker. 1970. The effect of oxytocin on fertility in female chickens and on sperm motility. Poultry Sci. 49: 810-812.

Hunsaker, W. G., J. R. Aitken, and G. S. Linblad. 1956. The fertilizing capacity of fowl semen as affected by time and temperature of storage. Poultry Sci. 35: 649-653.

Johnson, A. J. 1954. Artificial insemination and the duration of fertility of geese. Poultry Sci. 33: 638-640.

Johnston, N. P., and J. E. Parker. 1970. The effect of time of oviposition in relation to insemination on fertility of chicken hens. Poultry Sci. 49: 325-327.

Jull, M. A. 1947. Raising turkeys, ducks, geese, gamebirds. McGraw-Hill Book Co., Inc. New York, London.

Kamar, G. A. R. 1958. The collection of cock's semen without milking the copulatory organ. Poultry Sci. 37: 1382-1385.

_____. 1960. The influence of semen characteristics on hatching results of chicken eggs. Poultry Sci. 39: 188.

Kamar, G. A. R., and M. A. A. Razik. 1973. The relationships between semen characteristics and hatching results of turkeys. in Atti del VII Simposio internazionale di Zootecnia, Milano, 15-17 Aprile 1972, cited in A. B. A. 42: 528, 1974.

Kammerer, D. M., R. E. Moreng, H. D. Muller, and H. W. Hobbs. 1972. Turkey semen evaluation for fertility prediction. Poultry Sci. 51: 77-82.

Kashiwabara, T. 1965. Size of sperm head and of red blood cells in the domestic fowl. Poultry Sci. 43: 411-414.

Kinney, T., and R. E. Burger. 1960. A technique for the insemination of geese. Poultry Sci. 39: 230-232.

Kovacs, E. [Koplik, E.] 1972. The effect of calcium and phosphorus levels in the diet on spermatogenesis in the gander. Baromfiipar 19(1): 1-5, cited in A. B. A. 41: 634, 1973.

Landauer, W. 1961. The hatchability of chicken eggs as influenced by environment and heredity. Connecticut Agricultural Experiment Station Monograph No. 1.

Lamoreux, W. F. 1943. The influence of different amounts of illumination upon the production of semen in the fowl. J. Expt. Zool. 94: 73-95.

Lepore, P. D., and H. L. Marks. 1966. Intravaginal insemination of Japanese quail: factors influencing the basic technique. Poultry Sci. 45: 888-891.

Lorenz, F. W., and I. M. Lerner. 1946. Inheritance of sexual maturity in male chickens and turkeys. Poultry Sci. 25: 188-189.

Lorenz, F. W., N. E. Wilson, and V. S. Asmundson. 1955. Relation of frequency of collection to amount of semen obtained from turkey males. Poultry Sci. 34: 634-639.

_____. 1956. Effect of long intervals between collections on semen production of turkey males. Poultry Sci. 35: 823-824.

Maatsch, I., and U. Beyerbach. 1971. Uber die instrumentelle samenübertragung bei einem wanderfalkenpaar. Der Prakt. Tierarzt 4: 140-141.

Malstrom, M. V. 1943. Factors influencing fertility in the domestic fowl. M.Sc. Thesis, Univ. Conn.

Marks, H. L.; and P. D. Lepore. 1965. A procedure for artificial insemination of Japanese quail. Poultry Sci. 44: 1001-1003.

McCartney, M. G. 1956. Relation between semen quality and fertilizing ability of white Holland turkeys. Poultry Sci. 35: 137-141.

McCartney, M. G., V. D. Chamberlain, R. D. Carter, and J. W. Wyne. 1958. Effect of frequency of semen collection on fertility, hatchability and spermatozoa in the turkey. Poultry Sci. 37: 363-366.

McDaniel, G. R., and J. V. Craig. 1959. Behavior traits, semen measurments and fertility of white leghorn males. Poultry Sci. 38: 1005-1013.

_____. 1962. Predicting male fertilizing capacity in high and low fertility strains of chickens. Poultry Sci. 41: 866-869.

Mimura, H. 1941. Studies on the mechanism of travel of spermatozoa through the oviduct in the domestic fowl. J. Dept. Agric. Kyushu Imperial Univ. 6: 167-259.

Molnar, J., E. Kovacs, and Z. Sasvari. 1971. Influence of Ca level of feed on semen production in ganders. Baromfiipar 18(6): 269-276, cited in A. B. A. 42: 528, 1974.

Moore, O. K., and T. C. Byerly. 1942. Relation of time of insemination to percent fertility. Poultry Sci. 21: 253-255.

Munro, S. S. 1938. The effect of dilution and density on the fertilizing capacity of fowl sperm suspensions. Can. J. Res. D. 16: 281.

Mymrin, I. A., A. Bondarenko, and G. Krivich. 1972. Reproductive performance of turkeys and semen quality. Ptitsevodstvo 22(3): 15-17, cited in A. B. A. 41: 97, 1973.

Nestor, K. E., and K. I. Brown. 1971. Semen production in turkeys. Poultry Sci. 50: 1705-1712.

Nishiyama, H., K. Ogawa, and Y. Nakanishi. 1971. Studies on the artificial insemination in the domestic fowl. III. Sperm concentration of semen at collection and sperm quality of the semen. Memoirs of the Faculty of Agriculture, Kagoshima University 8(1): 355-366, cited in A. B. A. 41: 634, 1973.

Ogasawara, F. X., and R. Huang. 1963. A modified method of artificial insemination in the production of chicken-quail hybrids. Poultry Sci. 42: 1386-1392.

Owen, R. D. 1941. Artificial insemination of pigeons and doves. Poultry Sci. 20: 428-431.

Parker, J. E. 1945. Relation of time of day of artificial insemination to fertility and hatchability of hens' eggs. Poultry Sci. 24: 314-317.

Parker, J. E., and G. H. Arscott. 1965. The effect of time of insemination on fertility of hens' eggs. Poultry Sci. 44: 1405-1406.

Parker, J. E., and W. H. McCluskey. 1964. The effect of the length of daily light periods on the volume and fertilizing capacity of semen from male chickens. Poultry Sci. 43: 1401-1405.

Parker, J. E. and B. J. McSpadden. 1943. Seasonal variation in semen production in domestic fowl. Poultry Sci. 22: 142-147.

Penquite, R., W. A. Craft, and R. B. Thompson. 1930. Variation in activity and production of spermatozoa by white leghorn males. Poultry Sci. 9: 247-256.

Pingel, H. 1972. AI in duck breeding. Wissenschaftliche Zeitschrift der Humboldt-Universitat zu Berlin. Mathematische-Naturwissenschaftliche Reihe 21(2): 179-181, cited in A. B. A. 41: 634, 1973.

Porter, R. D., and S. N. Wiemeyer. 1970. Propagation of captive American kestrels. J. Wild. Mgmt. 34: 594-604.

Quinn, J. P., and W. H. Burrows. 1936. Artificial insemination in fowls. J. Hered. 27: 31-37.

Rowan, W. 1928. Reproductive rhythm in birds. Nature 122: 11.

_____. 1938. Light and seasonal reproduction in animals. Biol. Rev. 13: 374-402.

Saeki, Y. 1960. Crooked-necked spermatozoa in relation to low fertility in the artificial insemination of fowl. Poultry Sci. 39: 1354-1361.

Saeki, Y., and K. I. Brown. 1962. Effect of abnormal spermatozoa on fertility and hatchability in the turkey. Poultry Sci. 41: 1096.

Sampson, F. R., and D. C. Warren. 1939. Density of suspension and morphology of sperm in relation to fertility in the fowl. Poultry Sci. 18: 301-307.

Schwab, R. G. 1970. Light-induced prolongation of spermatogenesis in the European starling, Sturnus vulgaris. Condor 72: 466-470.

Shaffner, C. S., and F. N. Andrews. 1948. The influence of thiouracil on semen quality in the fowl. Poultry Sci. 27: 91-102.

Sharma, R. P., and N. S. Sidhu. 1971. Studies of genetic effects on the spermatozoan head in poultry (Gallus domesticus L.). Indian Poultry Gazette 55(3): 75-82, cited in A. B. A. 41: 213, 1973.

Siegel, S. 1956. Nonparametric statistics: for the behavioral sciences. McGraw-Hill Book Co., Inc., New York.

Sinha, S. P. 1971. Breed differences in the sizes of sperm head and red blood cells in the domestic fowl. Indian Poultry Gazette 55(2): 59-64, cited in A. B. A. 41: 213, 1973.

Smyth, J. R., Jr. 1968. Poultry. in Perry, E. J. (ed.), The artificial insemination of farm animals. Rutgers Univ. Press, New Jersey. pp. 258-300.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York.

Temple, S. A. 1972. Artificial insemination with imprinted birds of prey. Nature 237: 287-288.

_____. 1972. Chlorinated hydrocarbon residues and reproductive success in eastern North American merlins. Condor 74(1): 105-106.

Van Drimmelin, G. C. 1951. Artificial insemination of birds by the intraperitoneal route. A study in sex physiology of pigeon and fowls with reports upon a modified technique of semen collection and a new technique of insemination and observations on the spermatozoa in the genital organs of the fowl hen. Onderstepoort J. Vet. Res. Supp. No. 1.

Verma, O. P., and F. L. Cherms. 1964. Observations on the oviduct of turkeys. Avian Dis. 8: 19-26.

Warren, D. C., and C. L. Gish. 1943. The value of artificial insemination in poultry breeding work. Poultry Sci. 22: 108-117.

Watanabe, M., and Y. Sugimori. 1957. Studies on the artificial insemination in ducks. Zootech. e. Vet. 12: 119, cited in A. B. A. 25: 318.

Wentworth, B. C., and W. J. Mellen. 1963. Egg production and fertility following various methods of insemination of Japanese quail. J. Reprod. Fertil. 6: 215-220.

Wheeler, N. C., and F. N. Andrews. 1943. The influence of season on semen production in the domestic fowl. Poultry Sci. 22: 361-367.

Wilcox, F. H. 1958. The effect of dilution and concentration of chicken semen on fertility. Poultry Sci. 37: 1356-1362.

Willoughby, E. J., and T. J. Cade. 1964. Breeding behavior of the American kestrel (sparrow hawk). Living Bird 3: 75-96.

Wolfson, A. 1959. The role of light and darkness in the regulation of spring migration and reproductive cycles in birds. In Withrow, R. B., Photoperiodism and Related Phenomena in Plants and Animals. Amer. Assoc. Adv. Sci., Washington, D. C., pp. 679-716.

Wyne, J. W., M. G. McCartney, R. D. Carter, and V. D. Chamberlain. 1959. Relation of time of insemination to fertility in the turkey. Poultry Sci. 38: 828-831.

APPENDIX

TABLE 1

Analysis of Variance of Egg-laying Under Isolated and Exposed Conditions[a]

Source	df	SS	MS	F
Year	1	826.62	826.62	2.28
Treatment Combination	3	1742.43	580.81	1.60
Pen Design	1	99.41	99.41	0.27
Mating	1	589.62	589.62	1.63
Pen Design X Mating	1	1053.41	1053.41	2.91
Error	3	1087.83	362.61	
C.V. = 28.6%				

a = data transformed by arc-sine transformation

* Significant at 0.05 level
** Significant at 0.01 level

TABLE 2

Analysis of Variance of the Per Cent AI and NM Female Kestrels Laying Fertile Eggs[a]

Source	df	SS	MS	F
Year	1	46.56	46.56	0.44
Treatment Combination	3	1357.93	452.64	4.24
Clutch	1	355.64	355.64	3.33
Mating	1	288.24	288.24	2.70
Clutch X Mating	1	714.04	714.04	6.69
Error	3	320.23	106.74	
C.V. = 15.28				

a = data transformed by arc-sine transformation

* Significant at 0.05 level
** Significant at 0.01 level

TABLE 3

Analysis of Variance of Fertility Between AI and NM Female Kestrels[a]

Source	df	SS	MS	F
		Per Cent Fertile From AI		
Year	1	11.63	11.63	0.05
Mating	1	45.43	45.43	0.18
Error	1	260.18	260.18	
C.V. = 29.7%				
		Per Cent Fertile		
Year	1	1.22	1.22	.00
Mating	1	.01	.01	.00
Error	1	339.85	339.85	
C.V. = 35.8%				

a = data transformed by arc-sine transformation

* Significant at 0.05 level
** Significant at 0.01 level

TABLE 4

Analysis of Variance of Hatchability of Fertile Eggs of AI and NM Female Kestrels[a]

Source	df	SS	MS	F
Year	1	680.11	680.11	9.36
Treatment	2	233.31	116.66	1.61
Error	2	145.40	72.70	
C.V. = 14.2%				

a = data transformed by arc-sine transformation

* Significant at 0.05 level
** Significant at 0.01 level

TABLE 5

Correlations for Duration of Fertility and Semen Characteristics

Spearman Corr. Coeff. / Prob > |R| Under $H_0: \rho = 0$ / No. Obs.

	Semen Volume	Sperm Concentration	Sperm Count Per Ejaculate	Semen Appearance	Per Cent Contamination
Per Cent Fertility	-0.595238 0.1178 8	-0.443122 0.2713 8	-0.642857 0.0839 8	-0.102869 0.8025 8	0.378394 0.5944 7
Per Cent Hatchability	-0.392823 0.3371 8	0.030874 0.9404 8	0,012276 0.9756 8	-0.530371 0.1747 8	-0.254898 0.5848 7
Duration of Fertility	0.000000 1.0000 8	0.610789 0.1060 8	0.238095 0.5745 8	-0.205738 0.6282 8	0.198206 0.6710 7

TABLE 6A

Correlations with Age, Weight and Semen Characteristics

Corr. Coeff. / Prob > |R| Under H_0: $\rho=0$ / No. Obs.

	Sperm count per Ejaculate	Concentration of Spermatozoa	Semen Volume	Per Cent Successful Massages	Body Weight
Age	0.488766* 0.0376 18	0.265415 0.2871 18	0.566159* 0.0137 18	-0.301984 0.2215 18	0.836980** 0.0001 18
Body Weight	0.622528** 0.0059 18	0.490515* 0.0368 18	0.372590 0.1247 18	-0.048666 0.8419 18	

* Significant at 0.05 level
** Significant at 0.01 level

TABLE 6B

Correlations with Age, Weight and Semen Characteristics

Spearman Corr. Coeff. / Prob > |R| Under $H_0: \rho = 0$ / No. Obs.

	Sperm Motility	Semen Appearance	Per Cent Contamination
Age	0.266737 0.2846 18	-0.149296 0.5606 18	-0.150799 0.5695 17
Body Weight	0.327818 0.1816 18	0.077519 0.7573 18	-0.040516 0.8717 17

* Significant at 0.05 level
** Significant at 0.01 level

TABLE 7

Correlations with Weather Factors and Semen Characteristics

Corr. Coeff. / Prob > |R| Under H_o: $\rho = 0$ / No. Obs.

	Semen Volume	Sperm Concentration	Sperm Count Per Ejaculate	Sperm Motility	Per Cent Successful Massages
Temperature	-0.183412 0.6271 26	0.044905 0.8329 23	0.121407 0.5697 25	-0.125315 0.5572 25	0.098222 0.6115 30
Barometric Pressure	-0.126168 0.5457 26	0.142956 0.5218 23	-0.054763 0.7905 25	-0.093985 0.6590 25	0.042860 0.8167 30
Per Cent Possible Daily Sunshine	-0.070828 0.7305 26	0.197727 0.6313 23	0.116950 0.5840 25	-0.017559 0.9313 25	0.229267 0.2209 30

* significant at 0.05 level
** significant at 0.01 level

TABLE 8

Correlations with Fertility, Hatchability and Semen Characteristics

Spearman Corr. Coeff. / Prob > |R| Under $H_0: \rho = 0$ / No. Obs.

	Semen Volume	Sperm Concentration	Sperm Count Per Ejaculate	Sperm Motility	Semen Appearance	Per Cent Contamination
Per Cent Fertility	0.532566 0.0587 13	0.474043 0.0990 13	0.650913 0.0154 13	0.181185 0.5593 13	0.188185 0.5900 13	0.262417 0.6101 13
Per Cent Hatchability	-0.422156 0.1693 12	-0.160409 0.6229 12	-0.484024 0.1082 12	-0.318152 0.3144 12	-0.220562 0.5033 12	0.109370 0.7337 12

* significant at 0.05 level
** significant at 0.01 level

TABLE 9A

Correlations Among Semen Characteristics

Corr. Coeff. / Prob > |R| Under $H_0: \rho = 0$ / No. Obs.

	Sperm Count Per Ejaculate	Concentration Of Spermatozoa	Semen Volume
Per Cent Successful Massages	-0.081700 0.7455 18	0.125172 0.6257 18	-0.510642* 0.0288 18
Semen Volume	0.288277 0.2448 18	-0.015776 0.9491 18	
Concentration of Spermatozoa	0.649364** 0.0038 18		

* significant at 0.05 level
** significant at 0.01 level

TABLE 9B

Correlations Among Semen Characteristics

Spearman Corr. Coeff. / Prob > |R| Under $H_0: \rho = 0$ / No. Obs.

	Per Cent Contamination,	Semen Appearance	Sperm Motility
Per Cent Successful Massages	-0.059041 0.8163 17	0.314156 0.2020 18	-0.024896 0.9187 18
Semen Volume	-0.377451 0.1322 17	-0.457172 0.0539 18	0.216830 0.6088 18
Concentration of Spermatozoa	0.106683 0.6858 17	0.097057 0.7028 18	0.587293* 0.0101 18

* significant at 0.05 level
** significant at 0.01 level

TABLE 9C

Correlations Among Semen Characteristics

Spearman Corr. Coeff. / Prob > |R| Under $H_o: \rho = 0$ / No. Obs.

	Per Cent Contamination	Semen Appearance	Sperm Motility
Sperm Count Per Ejaculate	-0.188725 0.5257 17	-0.098039 0.7001 18	0.677336** 0.0023 18
Sperm Motility	-0.391171 0.1174 17	-0.403717 0.0935 18	
Semen Appearance	0.458333 0.0616 17		

* significant at 0.05 level
** significant at 0.01 level