ABSTRACT

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THE TRANSPORT OF AVIAN SPERM CELLS IN THE FEMALE REPRODUCTIVE TRACT

Labelling spermatozoa would be a valuable asset in studying competitive sperm transport between two individuals within a single female. One twentieth mg of fluorescein isothiocyanate (F.I.T.C.) per 2.00×10^9 sperm cells proved to be effective in irreversibly marking spermatozoa.

It has been reported that the utero-vaginal (UV) junction serves as a sperm barrier and sperm storage area within the female tract. Birds inseminated following the surgical removal of this tissue and birds inseminated past this area showed high fertility accompanied by a long duration. Hens inseminated with semen incubated with UV homogenate had lower fertility and duration.

In comparing low (RIR) and moderate (SCWL) fertility birds, more sperm reached the UV junction from the SCWL birds than from the RIR when equal numbers of each were inseminated in a single female. These SCWL birds consistently sired more offspring when in competition with the RIR birds.

Rooster sperm reached the UV junction prior to turkey sperm following heterospermic inseminations of the two species.

THE TRANSPORT OF AVIAN SPERM CELLS IN THE FEMALE REPRODUCTIVE TRACT

by

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1. INTRODUCTION

Over the past few years, experiments have been designed to determine whether the female possesses any selective mechanism in determining which sperm will ascend the oviduct and participate in fertilization. It has been observed that following mixed inseminations, one male can be much more successful in the number of progeny it produces than another. In this competitive fertilization, the question that is being asked is whether the female determines the offspring ratio or whether the nature of the sperm is responsible.

The ability to mark sperm would be a valuable asset in the study of sperm transport and storage. With the identification of sperm from one individual or species, sperm selectivity or competition between two individuals or species within a single female could be determined.

After sperm recovery, one could determine whether the ratio of offspring corresponded to the number of sperm cells from each male recovered at the site of fertilization. Possibly a more qualified assessment could then be made as to which member of the pair (i.e., the female or the male) is responsible for any discrepancies in the ratio of

offspring.

The female tract does limit the number of sperm that can possibly reach the site of fertilization. In mammals, the cervix, utero-tubal junction and isthmus are barriers. In the avian species it is the utero-vaginal junction and perhaps the utero-isthmal junction.

Among the questions which have not been answered but which would help to clarify the function of the oviduct are:

1) the role of the utero-vaginal junction in sperm transport and fertility;

2) the role of the utero-vaginal junction in the quality of offspring produced;

3) whether there is some mechanism selecting intra-species sperm moving to the site of fertilization (or sperm from closely related males); and,

4) whether the utero-vaginal junction enhances <u>in vivo</u> sperm viability?

The work to be reported here will have bearing on these points.

2. LITERATURE REVIEW

2.1. In Vivo Sperm Transport and Storage in the Bird

Early workers found that chickens could produce fertile eggs up to 20 days after separation from the male (Crew, 1926). However, this ability is not unique to birds. Certain bats can store sperm for five months (Wimsatt, 1944), dwarf chamelons for six months (Atsatt, 1953), the green anole for at least seven months (Fox, 1963), some viviporous fish for one year (Hartman, 1939), the common turtle for four years (Ewing, 1943), the snake for six years (Haines, 1940), and the queen bee for seven years (Bishop, 1920).

Ivanov (1924, cited by Van Drimmelen, 1946) believed that the sperm ascend the tract and enter both the ripe and unripe follicles. Spermicidal agents placed in the female tract did not prevent the long duration of fertility (Ivanov, 1924, cited by Van Drimmelen, 1946 ; Walton and Whetham, 1933); thus it was theorized that the sperm were not stored in the lumen of the tract. Walton and Whetham (1933) did express the idea that sperm storage might be accomplished in the folds of the oviduct.

2.1.1. The Role of the Infundibulum and Utero-vaginal junction

Van Drimmelen (1946) reported observing "spermnests" in the infundibular region of the chicken tract which could account for the long duration of fertility. These nests were located in shallow crypts in the caudal portion of the chalaziferous region. The passage of the egg down the oviduct could cause the expulsion of these sperm which are then available to fertilize the oocyte. Attempts to find sperm nests in the turkey's infundibular region have failed (Verma and Cherms, 1964).

Sperm tubules in much greater numbers have been reported in the utero-vaginal (UV) junction areas of both chickens (Fujii and Tamura, 1963; Bobr et al., 1964a; Takeda, 1967) and turkeys (Verma and Cherms, 1964). Sperm have been found to be deeply embedded among the microvilli at the distal end of the tubule (Van Krey et al., 1967).

The relative significance of the two storage areas has not been determined. The UV junction, besides storing sperm, acts as a limiting agent to sperm ascending the tract. Gertner (1963) reported on the structure of the sphincter area in the goose. Grigg and Skaller (1958) postulated that "an exaggeration of the restrictive function of the utero-vaginal junction" can cause infertility.

They reported that three hens which were sterile by conventional A.I. showed 90% fertility following intraperitoneal inseminations.

Dead sperm inseminated directly into the uterus would reach the infundibulum at the same rate and in the same number as would live. The limiting capabilities of this junction were readily demonstrated; .003-.03% of the sperm reached the infundibular region following intravaginal inseminations and .68-1.3% following intra-uterine inseminations (calculated from the work of Allen and Grigg, 1957).

Schindler et al. (1967) found that the infundibular glands are capable of maintaining viable sperm at least equal to the length of time observed in the UV glands. Inseminations past the UV junction result in a higher fertility accompanied by a longer fertile period (Takeda, 1966; Van Krey et al., 1966). Intra-uterine inseminations from low fecundity cocks result in moderate fertility (Ogasawara et al., 1966). Following these inseminations, nearly all the stored sperm are located in the infundibular region with little or no sperm in the UV tubules (Takeda, 1966; Van Krey et al., 1966). However, with deep inseminations, using both low (Ogasawara et al., 1966) and high

fecundity males (Van Krey et al., 1966), there is an increase in the incidence of pre-ovipositional embryonic mortality.

2.1.2. Sperm Release and Transport

It was found that dead sperm and charcoal powder reach the infundibulum as quickly as live sperm following intra-uterine inseminations (Mimura, 1939; Saeki et al., 1964). Mimura (1941) claimed that the ciliary movement within the tract could not account for the sperm transport, as Parker (1931) had so strongly suggested, and postulated oviduct motility as the major contributing factor.

In a study of tract motility, Chen and Hawes (1970) found the infundibulum to maintain the highest activity with a steady decline towards the vaginal end. They found that the UV area has the lowest contractility index and therefore suggested that it might act as a barrier to sperm movement from the vagina to the uterus.

Mimura (1939,1941) reported rapid transport of sperm post-oviposition, observing sperm at the site of fertilization 26 minutes after intra-vaginal inseminations. Saeki et al. (1963) found labelled sperm 16 minutes post-intra-vaginal insemination, whereas Donovan et al. (1969b) reported that sperm travelled the length of the oviduct

in 5-15 minutes, with peak numbers in the infundibulum at 60 minutes and an increase at the UV junction between 5-180 minutes.

An egg in the upper part of the oviduct hinders sperm transport in chickens (Mimura, 1939; Bobr et al., 1964b)and in turkeys (Howarth, 1971). Bobr et al. (1964b) suggested that the albumen does not allow the sperm to pass. A hard shelled egg has no effect on sperm transport (Mimura, 1939), although workers have reported decreased fertility when inseminations are performed using females with hard-shelled uterine eggs (Moore and Byerly, 1942; Parker, 1945).

Sperm release from the UV area was believed triggered by oviposition for it was at this time that most of the sperm were found in the lumen of the oviduct (Mimura, 1939; Bobr et al., 1964b). The sperm would then ascend the tract, and fertilize the oocyte or be stored in the infundibular region (Takeda, 1967). Secretory granules have been associated with the UV glands (Gilbert et al., 1968a)and they may be instrumental in causing sperm movement into the lumen.

On the other hand, workers have indicated that sperm can be released without oviposition and ovulation (Burke et al., 1969) and that sperm are constantly present

in the tract during the fertile period (Burke and Ogasawara, 1969).

2.2. Sperm Transport in Other Species

It is generally agreed that sperm transport in mammals is also due to tract contractions as suggested by Heape (1898).

The rate of movement for sperm to reach the oviduct post-insemination has been calculated as two minutes in the rat (Blandau and Money, 1944) and cow (VanDemark and Moeller, 1951), eight minutes in the ewe (Mattner and Braden, 1963), fifteen minutes in the mouse (Lewis and Wright, 1935), and thirty minutes in the hamster (Chang and Sheaffer, 1957) and human (Rubenstein et al., 1951).

When a sufficient volume (at least 20 cc) of semen is inseminated in sows, the number of sperm reaching the oviduct is directly related to the concentration of sperm in the semen (Baker, 1965). Pitkjanen (1958) reported that semen reached the sow's oviduct in an average of 14.7 minutes after natural double mating, 17.1 minutes after single mating, 41.6 minutes after artificial insemination, 14.9 minutes in sows mated naturally at the beginning of oestrus, 11.0 minutes when mated 24 hours after the onset of oestrus, and in 21.6 minutes when

mated 48 hours after oestrus.

First et al. (1968) found that 60% of spermatozoa inseminated in a sow was not recoverable from the uterus in 15 minutes, and that 75% was not recoverable after two hours. Rigby (1966) observed that the number of sperm in the oviduct increases until 12 hours after insemination, after which the tubal population is directly related to the number of spermatozoa at the utero-tubal junction. He recovered few sperm from the sow's oviduct killed six hours post-insemination but the number of sperm increased to 10,000, 12-24 hours post-insemination. However, in First's experiment, the number of spermatozoa in the oviduct remained unaltered from 1/4 to 24 hours after insemination.

Of the sperm remaining after the initial loss, the majority persist in a reservoir at the utero-tubal junction. In sows which were inseminated 24 hours after the onset of oestrus, this reservoir remained relatively unchanged for 24 hours and then gradually disappeared over the next 48 hours (Rigby, 1966).

It is unknown whether there is a selective mechanism determining the number of sperm entering the oviduct. First (1968) reported that dead sperm will ascend the tract in the sow as readily as live sperm and that

there was no significant difference in the number of sperm recovered from the oviduct of the two types. However, earlier reports in sheep (Mattner, 1963b) have indicated that live sperm will enter the oviducts more readily than will dead.

Mattner (1963a) observed that in ewes, cows, and goats, the cervix serves as a reservoir for sperm. There is a rapid movement of sperm up the tract in the first 15 minutes (this is due to uterine contraction) which is followed by a prolonged migration. Large numbers of spermatozoa remain in strands of mucus located between the microvilli and in the tubular or simple saccula cervical glands 16-25 hours after mating.

Mattner and Braden (1966) found that the path taken by the sperm is towards the wall of the cervix. Dead spermatozoa, however, were recovered only in the central area of the lumen of the cervix and none passed through the cervical mucus to the cervical mucosa. They also found that the utero-tubal junction prevents rapid passage of sperm into the fallopian tubes after recovering only 3,600-14,000 sperm from the oviducts of ewes killed 15 minutes - 24 hours post-insemination. This led Mattner (1963b) to suggest that the cervix, utero-tubal junction, and the motility of the spermatozoa themselves, are all responsible for the maintenance

of a continuous progression of spermatozoa from the reservoir (cervix) to the site of fertilization.

In the rabbit, it is also the cervix and the utero-tubal junction that are responsible for a reduction in sperm number. Braden (1953) calculated that 2.5% of sperm cells cross the cervix; .625% that reach the utero-tubal junction enter the oviduct, and 25% of these get to the ampulla. That is, .004% (1 out of 25,600) sperm inseminated reach the site of fertilization.

2.3. The Environment of the Female Tract and Sperm Viability

The mammalian sperm cell undergoes capacitation before it can fertilize the oocyte (Chang, 1951; Austin, 1951). During this process, a substance, decapacitation factor (DF), is removed from the sperm head increasing the membrane's permeability (Dukelow and Williams, 1967; Dukelow et al., 1967a).

Bedford (1969) observed that sperm require five to six hours exposure to fluid from the uterus and oviduct before they can fertilize the egg in rabbits. Eleven hours is needed in either the uterus or oviduct alone and 15 to 16 hours in the uterus if the uterus is separated from the oviduct at the utero-tubal junction. Evidence indicates

that capacitation relies mainly on secretions from the oviduct and minimizes the effect of the uterus. Once capacitation has occurred, the oviduct fluid is actually hostile toward sperm viability (Dukelow et al., 1967b).

Oviduct secretions greatly enhance respiration of mammalian sperm as reported in the ewe (Restall and Wales, 1966; Black et al., 1968; Iritani et al., 1969), rabbit (Hamner and Williams, 1963; Iritani et al., 1969), and boar (Schul II et al., 1966). Dukelow and Williams (1967) postulated that the increased metabolic rate decreases sperm survival time. It appears, therefore, that the tract limits the life span of the sperm through its post-capacitation effect and its causation of increased sperm metabolism.

In birds, however, some mechanism was developed for maintaining the fertilizing capabilities of the stored sperm. It has been suggested that the rooster's sperm do not undergo capacitation (but if it does, a very short time is required). Inseminations at approximately the time of ovulation has resulted in the first egg oviposited being fertile (Olsen and Nehrer, 1948). Howarth (1970) accomplished in vitro fertilization within 15 minutes, and the author indicated that the hen's reproductive tract is not needed for the maturing of sperm and fertilization. Dukelow et al. (1967a) failed to observe DP activity in

rooster seminal plasma postulating that there is no capacitation since DF is present in animals demonstrating capacitation.

However, as is the case with the female mammal, the bird's reproductive tract does increase the metabolic rate of spermatozoa. Hamner and Williams (1963) found a twofold increase in oxygen uptake of sperm incubated in the hen's oviduct. Albumen added to fowl sperm increased the respiration rate (Schindler and Lehrer, 1969). Magnum (Ogasawara and Lorenz, 1964; Van Krey et al., 1970), and uterus homogenates (Lehrer and Schindler, 1969) both augmented sperm respiration, although Lehrer and Schindler (1969) reported that appreciable amounts of albumen are needed along with the magnum.

Of major interest are the two storage areas. Interestingly, both the infundibulum (Ogasawara and Lorenz, 1964) and the UV junction (Van Krey et al., 1970) greatly increased sperm respiration. In fact, Schindler and Hurwitz (1966) reported that the UV junction, uterus, isthmus, and magnum are all capable of maintaining sperm motility in vivo for four hours but only the lower part of the isthmus and magnum could maintain it for 24 hours.

It is to be noted that at 41°C, the chicken's body temperature, sperm are rendered immobile (Munro, 1938;

Nevo and Schindler, 1968). One would then be inclined to theorize that under normal physiological conditions, sperm respire very little once stored in the chicken tract. However, Schindler and Lehrer (1969) reported no difference in the respiration rate of fowl sperm when measured at 37° or 41°C. It should be pointed out that homogenates from the UV junction, uterus, magnum, and infundibulum prolong the duration of motility and fertility of sperm (Lehrer and Schindler, 1969). However, these sperm suspensions were sampled and tested only four hours after collection which is not a long enough period to explain the long duration of fertility. Certainly one would think that there is some mechanism, as yet unidentified, acting in conjunction with the body temperature to not only inhibit motility but also decrease respiration and thus preserve the viability of the sperm.

2.4. Fertilizing Ability of Foreign Spermatozoa

Sperm from one species can be transported to the site of fertilization in another (Howe and Black, 1963; Coggins and Baker, 1968).

Although one species may show a certain level of fertility when inseminated with foreign sperm, this does not

indicate how the reciprocal mating will result. Fertility following the inseminations of goat with ram semen is quite common, whereas the reciprocal cross is usually unsuccessful (Bowerman and Hancock, 1963). In observing sperm transport in these two species, Hancock and McGovern (1968) found that sperm reached the Fallopian tubes in both ewes mated with goats and goats mated with ewes. They concluded, therefore, that low fertility cannot be explained by poor sperm transport.

A number of workers have studied the role of capacitation in the blocking mechanism of inter-species hybridization. Dukelow and Chernoff (1969) have reported that human and rhesus sperm can be at least partially capacitated in the rat, mouse, hamster, guinea pig, and rabbit uterus. Bedford and Shalkovsky (1967) indicated that capacitation of rabbit sperm can be initiated in the cat, rat, guinea pig, and ferret uterus. They postulated that capacitation involves two phases. The first phase is neither species nor organ specific. The second is limited to the female tract and is species specific.

Hammond and Walton (1929) failed to produce any offspring when domestic rabbits were inseminated with hare epididymal sperm. This cannot be explained by the inability of fertilization, for Adams (1957) reported 97.7% of the

ova from a domestic rabbit (Oryctolagus cuniculus) were fertile following inseminations with hare (Lepus europaeus) sperm and Chang et al. (1964) found 96% fertility using snowshoe hare. Apparently, the late morula or early blastocyst stage fails to develop (Adams, 1957) followed by a degeneration of the fertile ova (Chang et al., 1964).

Chang (1965) reported that ferret ova fertilized by mink sperm died about 22 days post-fertilization. This, he suggested, is about the time of changeover from the embryonic to the fetal stage. It appears then that many hybrid failures occur due to some obstacle just after fertilization.

High fertility can be obtained if the two different species are closely related; in crosses of distantly related species, fertility is rare. Coggins and Baker (1968) found that ram, boar, and human sperm would attach to but not penetrate rabbit ova.

Early workers reported avian hybrids; Ainsworth-Davis (1913) observed pheasant-fowl hybrids and Brentana (1914) peacock-guinea fowl hybrids. Ainsworth-Davis (1913) noted that most hybrid individuals die at an early age.

Warren and Scott (1935) reported finding fertile turkey-chicken hybrid eggs but concluded that it was difficult to hatch these eggs. Quinn et al. (1937) reported 20% fertility when crossing chicken males with turkey females.

Of the fertile eggs, 75% died the first day and all but one died by day 6 of incubation. When crossing turkey males and RIR females, 0.76% (5 out of 656 eggs) were fertile. Of this total, 80% (4 embryos) died on the first day and all had died by the third day. Olsen (1960) hatched 4 of 23 fertile eggs from turkey females which had been inseminated with chicken semen.

When inseminating Japanese quail with rooster semen, fertility was 7.8% of total eggs incubated; 0.4% hatched, while 6.0% died within the first 10 days and 1.4% died within the next 10 days (Wilcox and Clark, 1961).

Asmundson and Lorenz (1957) found reciprocal crosses of turkeys and pheasant did not differ consistently and averaged 37%. However, they did find higher fertility from chicken males to pheasant females than the reciprocal.

Contrary to the low fertility and hatchability usually reported in hybrid crosses, Watanabe and Ashida (1964) reported 100% fertility and hatchability of six eggs following the mating of a Korean Ringneck pheasant male to a Single Comb White Leghorn chicken.

From Steklenev's (1966) work, it is to be pointed out that the general tendency for low fertility cannot be attributed to the failure of sperm storage. He found that domestic cock sperm were stored 10 days in the reproductive tract of

guinea hens, 21 days in domestic hens, 25 days in pheasants, and 60 days in turkeys. Turkey sperm can remain 25 days in guinea hens and 35 days in domestic hens. There is no mention of the viability of these sperm.

Fertility can be improved with deep inseminations of foreign sperm. Kempenich-Pinto et al. (1970) found an increase in fertility in hens inseminated with turkey semen following intra-vaginal or intra-uterine inseminations vs conventional intra-vaginal inseminations. In the former hens, the sperm were stored almost exclusively in the infundubular glands with few sperm in the utero-vaginal region (Schindler et al., 1970). Six days after intra vaginal inseminations, few turkey sperm could be found; however, after deep inseminations, a large number were found but many had disintegrated. These workers concluded that the viability of the foreign sperm is a factor in the poor fertility and that the site of insemination can alleviate some of the problems.

The poor fertility from inter-species inseminations might be due to some barrier confronting the transport of the foreign sperm. Another possibility is that a greater number of sperm is required than generally needed for intra-species inseminations.

2.5. Competitive Fertilization

Heterospermic inseminations using mixed semen from more than one sire of the same species often results in unequal number of progeny from the respective sires. This phenomenon occurs although the number of sperm of each sire is maintained equal and has been reported in the mouse (Edwards, 1955), rabbit (Beatty, 1960; Napier, 1961), pig (Mellish, 1969), and cow (Beatty et al., 1969).

Similar male superiority has been demonstrated in the fowl; however, the results are sometimes inconsistent. Dunn (1927) found that the males closely related to the inseminated female produced a higher number of progeny when mixed with non-related males. Of 207 fertile eggs laid by single comb White Leghorn females, 97.6% were fertilized by their single comb sibs (rr), and the rest by rose comb Hamburg males (RR). In a smaller trial, 67% of 37 eggs were sired by the Hamburg males. The authors used natural mating and thus sperm numbers were not taken into account. These results might be explained by Crawford and Merritt (1963) and Buckland and Hawes (1968) who have shown that rose comb (RR) males are approximately 30% poorer in fertility than single comb (rr) sibs.

Bhatnagar (1963) reported when cocks of two breeds

were alternated, there was some indication of selective fertilization favouring the sperm from the male of the same breed as the female. Williams and McGibbon (1956) found that certain males tended to demonstrate a longer fertile period when mated to females of another inbred line than when mated to their own. Other workers did not find any affinity of sperm by the ova of the female of the same breed (Allen and Champion, 1955).

Atkinson et al. (1966) reported that Beltville Small White (BSW) turkeys sired 75% of the poults following heterospermic inseminations with Broad Breasted Bronze (BBB) males when BSW females were used. The BBB males did, however, give 13% better hatchability.

Buckland and Hawes (1968) reported a deficiency of pea (PP), rose (RR) and walnut (RRPP) comb offspring following heterospermic inseminations using semen from males of the above three genotypes mixed with semen from single (rr) comb males. They postulated that these differences were due to diploid gene action rather than haploid gene action of the sperm. Buckland et al. (1969) has since reported a deficiency of fumerase in the sperm of the rose comb cocks and this enzyme was found to be correlated with fertility.

Warren and Kilpatrick (1929) observed that replacing

the male in a chicken flock will result with the new male siring most of the chicks within three to five days with practically no overlap. They suggested that the sperm lose their tail in the female tracts and therefore the new sperm have an advantage. Fujii and Tamura (1963) also report viewing tailless sperm in the storage tubules.

Payne and Kahrs (1961) reported both a lack of and considerable overlap when fresh turkey sperm replaced the old male. The new sperm in competition with the stale sperm sired 71% of the poults the second day after insemination.

The sperm tubules in the UV junction fill with sperm starting with the most caudal ones (Verma and Cherms, 1965). The evacuation of the sperm is in the same order and thus the new sperm will be released first.

Allen and Champion (1955) suggested that selectivity might be based on the high degree of motility and a low incidence of abnormal sperm. A correlation was found between fertility and per cent dead sperm, motility, live density, and sperm respiration and no correlation with pH, density of all sperm, and volume of ejaculate (Cooper and Rowell, 1958). McDaniel and Craig (1962) reported a correlation between fertility and motility. They concluded that the appearance of the sperm is not associated with fertility. Boone (1968) found that fertility was correlated with per cent motile

sperm, concentration and sperm vigor and not correlated with volume or number of sperm. In a second trial he found no correlation with fertility and any of the five variables.

2.6. Methods of Labelling Spermatozoa

2.6.1. Radio-active Labelling

Workers have used both *in vivo* and *in vitro* labelling to study the physiology and anatomy of sperm. Radio-active studies have been used widely to follow spermatogenesis. Subcutaneous injections of p^{32} phosphate (Takeda *et al.*, 1968; Hupp *et al.*, 1961), adenine-8-C^{14} (Sirlin and Edwards, 1958; Foote and Koefoed-Johnson, 1959; Edwards and Sirlin, 1956) and thymidine-H^3 (Amann *et al.*, 1965) have proven successful in determining the rate of spermatid maturation. Radio-active male pronucleii were recovered in female mice 38 days after radio-active injections of the male, demonstrating that labelled sperm are capable of fertilization (Edwards and Sirlin, 1956). *In vitro* uptake of p^{32} by bull sperm has been reported by Bishop and Weinstock (1948) and Takeda *et al.* (1968).

Donovan *et al.* (1969a) reported that cock sperm and seminal plasma incorporates p^{32} into deoxyribonucleic acid *in vivo* and *in vitro*. Lorenz *et al.* (1950)

injected labelled Na_2HPO_4 subcutaneously in cocks and then determined p^{32} activity in the plasma, seminal fluid, and washed sperm. In vitro studies using p^{32} labelled sperm to determine the rate and number of sperm transported in the domestic chicken have been used (Allen and Grigg, 1957; Saeki et al., 1964; Donovan et al., 1969b).

Howarth (1971) labelled turkey sperm with ^{65}Zn and calculated the amount of radio-activity in various segments of the turkey oviduct using a whole body counter.

2.6.2. Uses of Fluorochromes

Hsia et al. (1969) has reported a surface active label in rabbits which can intercalate between lipid bilayers or lipoprotein fractions of plasma membranes. A less involved method of labelling has proven to be fluorochrome staining.

Van Demark et al. (1959) tested 14 fluorescent dyes for observing bovine sperm in opaque media. They concluded that coriphosphin gave best results and enchrysin 2, acridine orange, and akridingelb rated highly. The dyes were judged on fluorescence intensity when viewed under ultraviolet light. Their effect on sperm motility and rate was also taken into consideration.

These workers found no reduction in motility or rate of bull sperm when acridine orange was used. However, Van

Duijn (1960) reported this dye did have an adverse effect on sperm viability. He noted that the acridine orange labelled the bull sperm head homogenously and could therefore be useful in determining biometrical data of the external head shape and its area. Contrary to these findings, other workers have reported that acridine orange causes mammalian sperm to fluoresce brightly but the acrosome can be differentiated due to a different staining color under ultraviolet light (Bishop and Smiles, 1957; Bishop and Smiles, 1963).

Tetracycline HCl (T-HCl) binds to mammalian sperm causing the sperm to fluoresce distinctly under ultraviolet light. Capacitation causes the removal of the T-HCl and therefore one can show whether sperm has undergone this process by checking the sperm by fluorescence microscopy (Lauderdale and Ericsson, 1970).

Fluorescene is used mainly to label proteins (Coons et al., 1942). Riggs et al. (1958) prepared fluorescene isothiocyanate (F.I.T.C.) in a stable powder form. This fluorochrome has been used successfully in vitro in marking boar sperm and studying sperm transport in the pig's reproductive tract (Mellish, 1969; Baker and Degen, 1971). Mellish (1969) found F.I.T.C. to have no effect on the fertilizing ability of the sperm nor on the sperm's motility or rate. He did report that the sperm ceased movement the

instant they were struck by the ultraviolet light.

3. SPECIFIC STATEMENT OF PROBLEM

Various workers have proposed that one of the major functions of the female's reproductive tract is to limit the number of sperm reaching the site of fertilization. In the chicken, the structures reported as sperm barriers are the utero-vaginal and possibly the utero-isthmal junctions. One of these, the utero-vaginal junction, has been found to serve as a sperm storage area as well.

This work was conducted to:

1) obtain a suitable irreversible sperm marker to study sperm cell transport throughout the oviduct;

2) study the role of the utero-vaginal junction in sperm transport and storage by observing (a) the fertility in birds following the surgical removal of this tissue, and (b) the effect on fertility from mixing sperm with UV junction tissue homogenates; and

3) study sperm selectivity of the utero-vaginal junction in relation to inter- and intra-species inseminations.

4. MATERIALS AND METHODS

4.1. Experimental Birds and Management Practices

The males used in the various experiments were Rhode Island Reds (RIR), Single Comb White Leghorns (SCWL) and crosses between the two (RIR x SCWL). The females were RIR, SCWL, RIR x SCWL, Mutant Marker I (MMI), and S.C. Brown Leghorns (SCBrL). There were 24 males in each group at the start of the experiments; 4 SCWL and 1 RIR x SCWL died during the trials.

The SCBrL, RIR and SCWL have been maintained at Macdonald College as closed unselected pedigreed populations for 5, 8 and 12 generations, respectively. The RIR x SCWL were produced from the random mating of RIR males and SCWL females. The MMI is a synthetic line produced at Macdonald College in 1963 and maintained by mating females of this line to SCBrL males.

All birds were reared with sexes intermingled in conventional litter floor pens from hatching to 20 weeks of age. They were housed in individual cages at 20-22 weeks of age and subjected to 16 hours of artificial light. This was increased to 18 hours at 40 weeks of age and 20 hours at 52

weeks. The birds received conventional commercial ration for their appropriate age and this ration along with water were provided ad lib.

Day-old English Ringneck pheasants and William's mini-white turkeys were purchased from a commercial hatchery (used in Section 4.5.5.). The pheasants were managed in a manner similar to the chickens. Male and female turkeys were reared together in conventional floor pens until maturity. The males were then removed to individual pens, and the females remained together.

All birds used were in their first year of production or just reaching sexual maturity.

4.2. Insemination Procedures and Handling of Eggs

Semen was collected using the abdominal massage technique described by Burrows and Quinn (1935,1937). The males were ejaculated twice weekly throughout the experimental period to maintain a good quality and quantity of semen.

Intra-vaginal inseminations were carried out according to the procedure of Quinn and Burrows (1936) using 0.05 ml of pooled semen. In certain experiments this method was modified; these modifications will be described in the appropriate section. All inseminations were performed in

the afternoon since workers have shown that the highest fertility is obtainable at that time (Parker, 1945).

Eggs were collected daily for days 2-21 post-insemination, dated and marked by individual cage numbers. They were stored at 4.4°C for 4-7 days before being incubated in a model 2520 Jamesway Incubator. The incubator was managed according to the recommendations of the manufacturer.

All eggs were candled and classified as fertile, non-fertile, or dead embryos after 5-7 days of incubation. The eggs containing live embryos were returned to the incubator and later transferred to a hatching incubator at day 17 of incubation. The fertility percentage was based on the number of fertile eggs (includes dead embryos) relative to the total number of eggs laid from days 2-15. To determine the duration of fertility, all eggs were incubated and candled for 21 days. Only eggs from chickens laying at least 2 eggs in the first 7 days and 4 in the 14-day total were considered for statistical analysis.

The turkey females were kept in a floor pen and eggs could not be individually identified. Identical storage and incubation procedures were used with these eggs allowing for the additional week of incubation required for turkey eggs.

4.3. Trials Involving the Marking of Spermatozoa

4.3.1. Fluorochromes

This trial was conducted to determine whether fluorochromes could stain chicken sperm. Stock solutions (0.1%) of fluorescein isothiocyanate (F.I.T.C.), acridine orange, and tetracycline hydrochloride (T-HCl) were prepared in Wilcox diluent (Wilcox, 1958). Various levels of the resulting solutions were chosen for testing and added to the semen. Each mixture was stirred gently on a magnetic stirrer. The samples were checked periodically under an ultraviolet (UV) microscope for sperm fluorescence intensity. A reflected light microscope (400x) equipped with a mercury vapour lamp, exciter filter BG12 and barrier filter 53 and 44 was used.

4.3.2. Stained Spermatozoa in the Chicken's Reproductive Tract

The effect of various sections of the chicken's reproductive tract on the stained sperm was tested. F.I.T.C. or acridine orange was added to the semen and stirred until the sperm fluoresced brightly and distinctly. The semen was then spun at 400 R.P.M., the seminal plasma aspirated off, and the sperm re-suspended with Wilcox diluent to restore the original volume in each sample. In some cases,

interference was present from excess dye in the seminal plasma which obscured the cells. In such instances, the sample was re-spun and the procedure repeated.

Three birds were inseminated intravaginally with acridine orange stained sperm and three with F.I.T.C. stained sperm and treated as described above. Hens (RIR x SCWL) were then anesthetized with an i.v. injection of pentobarbitol sodium. The bird was placed on a dorsal recumbency, feathers were removed from the abdominal area, and this region was swabbed with 75% alcohol. An incision was made just posterior to the rib cage and to the left of the mid-line. A body retractor was placed into the incision and the entire reproductive tract was exposed a section at a time. Six areas of the tract were isolated with)) Catgut: the infundibulum, magnum, isthmus, uterus, UV junction, and vagina. Three hours later, the birds were killed, their reproductive tracts removed and each of the six sections was removed and placed in a separate evaporating dish. T-HCl was eliminated as a sperm marker. Reasons for this will be found in the results section. Each tract section was flushed into the dish using Wilcox diluent and the contents of the dish were transferred to 50 ml centrifuge tubes. These tubes were centrifuged at 1500 R.P.M. for 30 minutes (International Centrifuge, Universal Model UV), the supernatant aspirated off and the

residue examined for sperm. The sperm were checked for motility and fluorescence.

4.3.3. The Effect of F.I.T.C. on Fertility

To observe the effect of F.I.T.C. on fertility, 3 trials were performed. Three levels of F.I.T.C. were tested; .1 mg, .05 mg, and .025 mg per ml of semen (2.0×10^9 sperm cells). In the third trial .05 mg and .025 mg were tested. One-tenth ml of the dissolved dye was added to each sample which was then stirred gently. In addition to the stained sperm, 3 controls were used:

1. an immediate control inseminated immediately after semen collection;

2. a control in which the semen was kept for the 15-minute staining period at room temperature; and

3. a control which was kept for the staining time and was stirred as well.

In the first and second trials, the semen samples were divided into 6 equal aliquots and in the third 5 aliquots were used.

4.3.4. Attempts to Identify Sperm on the Mucosal Surface of the Female Tract

F.I.T.C. stained spermatozoa were placed directly on

different parts of an excised longitudinally cut chicken reproductive tract. Various sections were then placed on a thin transparent plastic and the samples were viewed under UV light. The light source was directed both from underneath and over the sample in an attempt to identify the sperm. Phase contrast microscopy was used as well in an effort to locate these sperm.

To determine if sperm could retain their fluorescence following histological treatment; F.I.T.C. stained sperm were dehydrated in ethyl alcohol and cleared in toluene.

4.3.5. Mixed Inter-Specific Inseminations Using F.I.T.C. Stained Sperm

Semen was collected from RIR males and RIR x SCWL crosses. Half of each of the samples was stained with .05 mg per 2 x 10^9 sperm cells per ml for 15 minutes. Each of the samples was spun, and the sperm resuspended to the original volumes as in Section 4.3.2. One-twentieth ml of RIR stained semen and .05 ml of RIR x SCWL unstained semen were inseminated within a single hen independently so as not to contaminate the unstained sample. The reciprocal cross was then carried out. Controls using stained and unstained RIR sperm within the same female and stained and unstained RIR x SCWL crosses within the same females were

similarly performed. The vagina, UV junction and infundubulum were then removed as described previously.

The recovery of the sperm was as follows. The lumen of the tissue was filled with Wilcox diluent until extended. It was then massaged gently to force sperm into the lumen. The catgut at one end was cut and the contents were transferred to an evaporating dish. The tissue was then cut longitudinally and stretched by fingertip manipulation. Wilcox diluent, by means of pressure from a syringe and needle,was used to rinse the tissue. The tissue was then scraped gently against the side of the dish in an attempt to force out additional sperm. The recovery of these sperm was similar to the method described earlier.

The recovered sperm were viewed under white light microscope. These same sperm were immediately checked under UV light to determine the number of fluorescing sperm. The percentage of stained sperm was then calculated.

4.3.6. Inter-Specific Inseminations and Competition

Sperm transport competition using turkeys vs RIR x SCWL and pheasants vs RIR x SCWL inseminated into SCWL females were tested. Numbers of sperm were kept equal and the procedure of mixing and staining the sperm was similar

to the method described earlier. However, due to a shortage of pheasant semen, no mixed pheasant and RIR x SCWL mixtures were used.

In a further study, the turkey and pheasant semen was inseminated into SCWL females from which the UV junction had been surgically removed to test the function of the sphincter as a barrier to foreign sperm. Intact females were used as controls. Inseminations were also made to determine the fertility of turkey x turkey matings.

4.4. The Effect of the Utero-Vaginal Junction on Fertility

4.4.1. The Removal of the UV Junction

Trial I

Twelve mature F_1 Rhode Island Red (RIR) x White Leghorn (WL) females (which were selected on the basis of a high rate of lay) were used for this work. The birds were anesthetized with an i.v. injection of 1.8 mg and an i.m. injection of 1.3 mg of pentobarbital sodium per 100 gm body weight.

The bird was then placed on a dorsal recumbency. Feathers were removed from the abdominal area and the exposed surface was swabbed with 75% alcohol. An incision (about 6 cm long) was made slightly to the left of and parallel to

the midline commencing just ventral to the vent. A body retractor was placed in the incision, and the uterus, UV junction and vagina were exposed. Fat and connective tissue were carefully pried away to allow for easier accessibility to this area. The vagina was then severed just posterior to the UV junction followed by the severing of the uterus just anterior to the junction. Both the vagina and uterus were grossly examined to determine that all UV tissue was removed. This was followed by an end-to-end anastomosis of the vagina and uterus using Blue Braided Dacron Polyester Fiber 6-0, TE-3/8 with a circle taper needle (Davis and Geck). The suturing was initiated on the dorsal side and worked laterally towards the right until about half the suturing was complete. New suturing was then started adjacent to the first knot on the dorsal side and worked laterally towards the left until it reached the point where the first suturing stopped. Continuous stitches were used. Before suturing of the muscle and skin layers, antibiotic powder (Ayerst) was dusted in the incision. Following surgery, each bird received an injection of 5 mg of diethyl stilbestrol (DES). Recovery was complete, in each case, the day of the operation.

To maintain proper oviduct function, the birds were given an i.m. injection of 5 mg of DES twice weekly for

three weeks. With each injection, the bird's vagina was inverted and any disintegrating ova found in the uterus were removed manually. As well, suturing material from a number of birds was removed at this time.

Trial II

Essentially the same procedure was followed with 9 pure-line SCWL females just prior to sexual maturity. However, 9 mm wound clips (Clay-Adams) were used on the skin layer of these birds; the clips being removed 4 days post-surgery.

Trial III

Experiment III was conducted using 30 pure-line SCWL females just prior to sexual maturity. Following the operation, the birds were injected with 5 mg of DES twice weekly for two weeks. Eight birds were re-operated for the removal of a hard-shelled egg from either the uterus or abdominal cavity.

Birds were maintained in individual cages and supplied with commercial laying ration and water ad lib.

Following the resumption of lay, the birds were inseminated with males of proven fertility (RIR x SCWL). All birds in these trials were subsequently killed and the tracts examined for UV tissue. Both gross examination and

histological studies were employed. The histological procedure on the tissue surrounding the operated area was as follows: (1) fixed in Bouin's solution, (2) dehydrated in ethyl alcohol, (3) cleared in toluene, and (4) embedded in paraffin wax.

The material was then sectioned at 7 microns, dehydrated, stained with hematoxylin, counterstained with eosin Y (.1%) and cleared through xylene.

4.4.2. The Effect of the UV Junction Homogenate on Fertility

The effect on fertility of semen incubated with UV junction homogenate for various lengths of time was tested. Mature SCWL females were killed and the UV junction was removed using a fine pair of iris scissors. The tissue was weighed and diluted 1:2 with Wilcox diluent. The mixture was then finely ground by means of a homogeniser (Virtis "23"). The homogenate was added to RIR x SCWL semen in a ratio of 1:2. A second sample of homogenate was centrifuged, the supernatant removed and added to whole semen in the same ratio (1:2). Similarly, Wilcox diluent was added to a third sample. Two controls were used and thus there were 5 samples in total. Four of these (all but one control) were incubated in a temperature regulated water-bath shaker

(Eberbach Corp.). The samples were shaken gently at 41°C for selected lengths of time as described below. The second control was maintained at room temperature for the same duration as the first four samples were incubated.

The females inseminated, RIR x SCWL and SCWL, were mated at random. In order to equalize the number of sperm cells per insemination, .067 ml of the diluted samples, and .05 ml of the undiluted samples were inseminated in each female.

Five separate trials were conducted. In trials 1, 2 and 3, the incubation time was 60, 90 and 30 minutes, respectively. In 1 and 2, no supernatant was used with the semen and in trial 3, the UV homogenate was omitted. In trial 4; 0, 60 and 90 minutes and in trial 5; 0, 30 and 60 minutes of incubation were tested.

Fertility per cent and duration of fertility were calculated in each trial.

4.4.3. Cannulation of the Uterus

This experiment was initiated to inseminate birds so that the sperm would avoid the UV junction.

Method I

The cannula used was made of polyvinyl chloride (Dural Plastics) with an O.D. of 2.5 mm, an I.D. of 1.5 mm

and a length of about 15 cm. Blue Braided Dacron Polyester Fiber 6-0 suturing material with a 3/8 circle taper needle (Davis and Geck) was attached to one of the ends of the cannula.

The uterus was exposed as in section 4.4.1. A small hole was made in the posterior section of the uterus by blunt dissection. The circle needle was passed into the lumen of the uterus through this opening, and manipulated gently using a hemostat to a point about 2 cm anterior to where it entered. The needle was then drawn through the uterine wall, the cannula eased into the uterus and positioned against the inner wall of the anterior end. The suturing material attached to the leading end of the cannula was drawn through the needle incision and used to stitch the cannula securely to the anterior wall. The cannula was then flushed with Wilcox diluent to assure an easy flow of liquid. The trailing end that was to remain outside the body was closed by heating to prevent contamination of the tract from the exterior. Suturing of the muscle and skin was completed about this end of the cannula which was secured to the skin surface to prevent the bird from pecking it.

Method II

The cannula used was the same as in method I, except

one end had a round flat polyvinyl circle about double the outside diameter of the cannula about it (it was held together by the melting of the two) and the other end was bevelled to a sharp point (See Figure 8).

The uterus was exposed as in section 4.4.1. A hollow tube, slightly larger in diameter than the cannula, and slit longitudinally, was inserted into the vagina. This tube was manipulated by means of both hands, i.e., one from the vaginal side and one probing within the body cavity, until it passed the UV junction and was seen to reach the uterus. The cannula, bevelled end first, was inserted into the tube and gently forced out through the uterine wall and into the body cavity. The hollow tube was removed by sliding the cannula through the longitudinal slit. This was required since the round knob on the end of the cannula would not allow it to pass through the tube. The cannula was then pulled through the small opening in the uterus until halted by the knob. No suturing was necessary here since the cannula was secured by the polyvinyl circle. The remainder of the procedure was similar to Method I.

Only one bird in Method I and five in Method II (all were SCWL) were operated. However, it was hoped that this technique could be used successfully in further studies as will be described.

These birds were inseminated via injecting semen through the cannula with the resumption of egg laying. Fertility per cent and duration of fertility were determined.

4.5. Competition Between Avian Males

4.5.1. Semen Comparison

Semen was collected twice weekly from RIR, WL, and RIR x SCWL males starting November 4, 1970 and continued until mid-May, 1971. Measurements were taken in semen volume and sperm cell number (by hemacytometer, American Optical Co.). Motility, rate, and abnormal sperm were noted subjectively.

There are a number of reasons the comparisons between the RIR and SCWL males were carried out. Firstly, it had been observed in past years in our laboratory and confirmed by experiments in August and September of 1970 that the RIR males had a low average fertility (Appendix Table I). In this sense, they should be ideal when in comparison to another breed in determining competitive or selective fertilization. As well, the SCWL carry dominant white (II) while the RIR are homozygous for the recessive allele for full color (ii). Thus, following heterospermic inseminations into a homozygous recessive female (ii), the offspring

from the two types of sires can be readily identified.

4.5.2. Heterospermic Inseminations

Four trials involving heterospermic inseminations from RIR (ii) and SCWL (II) males inseminated into MMI (ii) females were conducted. Two groups of females were inseminated with either RIR or SCWL semen and a third group was inseminated with a mixture of equal numbers of sperm from the two breeds. In total, 127 birds and 1141 eggs were used in the experiment.

Four additional trials were carried out using the female sibs of the RIR males. In trials 1, 2 and 3, the females were divided into four groups at random. One was inseminated with RIR semen, a second with SCWL semen, a third with equal numbers of sperm cells from each male and a fourth with twice as many sperm cells from the RIR males as from the SCWL males. In the fourth trial, five groups of females were used: in addition to the RIR and SCWL single inseminations, 2.5:1, 3:1 and 4:1 ratios of sperm cells in favour of the RIR were tested. One hundred and fifty-six females were inseminated from which 1451 eggs were incubated.

To establish the effect of inseminating females of the same strain, done in the previous set of experiments,

the trials were conducted using SCWL females. Similar combinations of inseminations were repeated as in the first set of experiments. RIR x SCWL males were used as a control for they were known to be of high fertility. Here, 63 hens were inseminated and 643 eggs were used.

A final trial was conducted to determine the fertility of the three groups of males in high fertility females (RIR x SCWL). The purpose of these trials was to use a type of female related equally to the two male lines. The trials were identical to the above with 72 females inseminated and 663 eggs incubated.

In the last two sets of experiments, the offspring could not be accurately identified and therefore only fertility per cent was noted. In all other trials, the percentage of sired chicks from each breed of males was determined.

The actual number of spermatozoa transported from RIR and SCWL males was determined within RIR x SCWL females, as described in section 4.3.5. Any differences in number of sperm cells might indicate discrepancies in the ratios of sired chicks. Five sections of the tract (vagina, UV junction, uterus, magnum, and infundibulum) were checked for sperm. Approximately 2 cm of the middle of the magnum was used. Three females were inseminated in each treatment for

a total of 27. All inseminations were done in the morning after an oviposition since Mimura (1939) and Bobr et al. (1964b) indicated that there is best sperm transport at this time. The birds were killed and scored for sperm number 30, 60, and 90 minutes post-insemination. The time required for the flushing of the different areas of the tract from death was 20-25 minutes per bird.

4.5.3. Inseminations Anterior to the UV Junction

In an attempt to inseminate past the UV junction and perhaps change the ratio of offspring produced, two methods were utilized: (1) cannulation of 12 MMI females as described in Method I, section 4.4.3; and (2) by injecting semen into the magnum of 27 MMI after surgically exposing this tissue as described in section 4.3.2. The semen combinations inseminated in both groups were identical to the inseminations used in the intact females.

4.5.4. Fresh vs Aged Spermatozoa

RIR females were divided into two groups; one being inseminated with RIR semen and the other with SCWL. Four days later, the group inseminated with RIR semen was re-inseminated with SCWL, and the reciprocal was carried out

in the second group. The experiments were conducted to see if the fresh sperm has an advantage and to determine the time required before the new males can fertile the oocyte.

4.6. Scoring of Sperm Numbers and Statistical Analyses

The number of drops in the test tube following centrifugation and aspiration of the tissue flushing was established by suctioning up the residue in a Pasteur Pipet and releasing it drop by drop (about 10 for the vagine and 2-4 for the other part of the tract). The residue was well mixed and one drop was placed on a microscope slide, and a coverslip on top of it. At 320x, each coverslip had approximately 104 columns. By counting a number of these at random (about 3 in the vagina, 10 in the UV junction, and 20-30 in the other parts) one can estimate the number of sperm recovered by the following formula:

$$\text{No. sperm observed} \times \frac{104}{\text{no. of rows counted}} \times \text{no. of drops}$$

In sections 4.3.5. and 4.5.2., the Chi-square test for heterogeneity (Snedecor, 1961) was used to determine if the ratio of sperm recovered or chicks from heterospermic inseminations deviated from the expected. Three methods were used to calculate the expected number of chicks:

1) based on the number of sperm from each group of

males, i.e., if equal numbers of sperm were pooled from each, there should be a 1:1 ratio of offspring;

2) based on per cent fertility, i.e., there should be equal number of progeny per group if the per cent fertility of the two groups is equal and the number of sperm is in a 1:1 ratio. If RIR and SCWL males are used (sperm in equal numbers) then the expected number of white chicks =

$$\frac{\text{\% fertility of SCWL}}{\text{\% fertility of SCWL + \% fertility of RIR}} \times \text{no. of offspring}$$

3) based on the per cent recognizable offspring, i.e., hatched, pips, and identifiable dead embryos. The expected observations are identical to the method employed in number 2 with fertility replaced by recognizable chicks.

For the analysis of variance of per cent and duration of fertility, a completely randomized design was used with the model: $Yij = \mu + \alpha i + \beta ij$

where Yij is the jth observation in the ith treatment,

μ is the effect due to the overall mean,

αi is the effect due to the ith level of the treatment,

βij is the error associated with the jth observation in the ith treatment.

In all cases, the analysis was conducted on equal number of observations per treatment, and the lowest number in each trial was used. Thus, certain birds were removed at random to equalize the number per group. On the tables

both figures will be presented, i.e., before and after the equalization of bird numbers. The latter value will be in parentheses.

Duncan's multiple range test was utilized to separate the means in the analyses where the factor means differed (Little, 1966).

5. EXPERIMENTAL RESULTS

5.1. The Marking of Spermatozoa

The three fluorochromes tested, F.I.T.C., acridine orange, and T-HCl, were all capable of staining sperm. Of the three, acridine orange proved to be the most effective in creating high sperm fluorescence intensity. The F.I.T.C. was adequate, i.e., the marked sperm were easily distinguished microscopically. The T-HCl stained sperm exhibited only faint fluorescence and as a result, T-HCl was eliminated from further experimentation as a possible sperm marker.

Although acridine orange was superior to the other two in terms of intensity, sperm stained with this dye lost their fluorescence after injection into and subsequent recovery from the female tract. On the other hand, F.I.T.C. stained sperm maintained their fluorescence and motility within the three-hour trial period in which the sperm were maintained, *in vivo*, in various parts of the tract.

After testing various levels of F.I.T.C., it was observed that .025-.1 mg of F.I.T.C. per ml of semen (2.00×10^9 sperm cells/ml) resulted in greater than 95% of the

sperm being stained within a five-minute incubation period. In order to assure maximum fluorescence for identifying the sperm following recovery from the female tract, 15 minutes of staining was required. The stained sperm could readily be identified by quickly scanning the oviduct flushings on the slide. Sperm which would otherwise have been obscured by debris were easily detected.

In the fertility trials involving the stained sperm, the .1 mg level had the lowest fertility in trial I ($P < .1$) (Table II) and in trial II this level also proved to be detrimental to fertility ($P < .01$) (Table III). The .1 mg level was thus eliminated and not used in trial III (Table IV). Both the .05 and .025 mg levels were comparable in fertility percentage to the immediate controls.

The non-stirred control (Control I) was consistently lower than the other two controls (Immediate and Control II) and the .05 and .025 mg levels of F.I.T.C. and in trial II differed significantly from the immediate control ($P < .01$). The stirred control (Control II) caused a lowered fertility in trial II as well ($P < .01$).

Stained sperm were then inseminated intra-vaginally into hens in lay. The birds were killed within 90 minutes, the reproductive tract excised, cut lengthwise, and placed under a U.V. microscope in an attempt to study sperm

transport by actually identifying individual cells. Slides were also prepared as described in section 4.3.4. to determine whether stained sperm could be identified in histological studies. Both techniques proved unsuccessful, and it was necessary to resort to flushing sperm from the tract as described in section 4.3.5.

Initial sperm recovery from intra-vaginally inseminated females using F.I.T.C. and acridine orange stained sperm is recorded in Table I. All F.I.T.C. stained sperm recovered (by the flushing method) within 90 minutes showed fluorescence. The acridine orange fluorescence was not maintained for any of the recovery periods.

5.2. The Role of the Utero-vaginal Junction

5.2.1. Fertility Following the Removal of the Junction

The effect of oviduct surgery on subsequent egg production can be seen in Table V. The females approaching sexual maturity in Trials II and III were more tolerant of the surgery than were the mature birds in Trial I. A gross examination was done on all birds which died or which did not resume egg production after approximately 90 days. In general, the ovary and tract had regressed to the stage of

an immature bird. However, some females continued to ovulate either into the body cavity or into the oviduct; the latter resulting in compaction of this organ. Some individual cases may be worthy of mention.

Trial I: Bird #1790 (died 243 days post-surgery) - The uterus was occluded with at least 3 ova. The magnum and isthmus were completely blocked with albumen-like material. The size of the oviduct appeared normal but regression of the ovary had occurred.

Trial II: Bird #1494 (died 95 days post-surgery) - Five distinct membrane covered eggs were present in the lower tract.

Bird #3377 (killed 137 days post-surgery) - The ovary appeared as in a functional laying female; 10 large follicles were present, 3 of which were approximately 20 mm in diameter. No ova were present in the body cavity. Although the bird had not laid an egg in over 100 days, the oviduct remained in a well developed state. The operated area was well healed and no U V tissue was observed (see Figures 4 & 5).

Trial III: Bird #424 (died 23 days post-initial-surgery due to oviduct hemorrhage while being re-operated) - The oviduct was normal in appearance. The ovary contained 7 large follicles, and 2 degenerating shelled eggs were

found in the body cavity.

The percentages of birds which died without resuming egg production were: Trial I - 42%; Trial II - 33.3%; Trial III - 23.3%. The average time of death was approximately 96 days post-surgery. Birds which did not resume egg production in Trial III were re-operated. The problem in these hens was not one of forming the hard-shelled egg, but, rather oviposition. Apparently the egg could not fit through the sutured area. Three re-operated birds had hard-shelled eggs in the abdominal cavity at this time; these eggs were removed.

Birds which produced fertile eggs in Experiments I and II were subsequently killed and the tracts examined for UV tissue. In all cases the tracts appeared normal, the suture was well healed and there was no gross evidence of the UV sphincter area. It should be noted that the characteristic curve in this area was absent. Histological studies did reveal some tubules. These were sparse and not concentrated in any one area.

In Trial I (Table V) the 4 birds which resumed egg production did so at 55, 87, 164 and 168 days respectively post-surgery. Due to the erratic pattern of production, females were inseminated on an individual basis as they came into lay. Following the first insemination, the 4 females

from Trial I laid from 1 to 4 fertile eggs each. One female remained fertile for as long as 14 days post-insemination. Only 1 of the original 4 hens continued to lay, and was inseminated for three subsequent periods. In two of these three periods, she produced 7 and 4 eggs which were 85.7 and 100% fertile, respectively.

The females from Trial II (Table V) were delayed in initiating egg production by 2-3 weeks but recovered much more quickly than the older females of Trial I. Table VI is included to show the individual variation exhibited by these birds both in egg production and fertility. One sham operated bird did not initiate production; the second did and was fertile for one of three insemination periods. In general, egg production was much lower than for normal birds of comparable age yet both duration and percentage of fertility were higher than might be expected in birds lacking the UV area for sperm storage.

The results of Trial III (Table V) show a decrease in time required to initiate egg production as compared with Trial II. Unfortunately, no sham operated controls were available in this trial and intact females were used as controls. In insemination period number 5, egg production improved to a level approaching that of the controls while fertility for periods 4 and 5 was increased over that of the

control.

Eggs from insemination periods 4 and 5 were allowed to hatch (Table VIII). From a total of 104 eggs incubated over these 2 periods, 83.6% were fertile; 47.1% (of fertile eggs) hatched, 17.3% were pips and 35.6% were dead embryos. It might be expected that hatchability would fall when sperm are stored in vivo for 14 days but a hatchability level of less than 50% is quite unusual. Comparable figures obtained from similar types of intact females in our laboratory average about 50% fertility over the 14-day post-insemination period. Hatchability of fertile eggs averages 82% while dead embryos and pips average 12 and 6%, respectively.

5.2.2. The Effect of UV Homogenate on Fertility

In all cases, non-incubated sperm combined with UV junction homogenate and sperm incubated for 30 minutes resulted in a consistent, but not significant decrease in fertility.

In Trials I and V (Tables IX and XIII) sperm incubated for 60 minutes with UV homogenate resulted in a lower fertility ($P < .05$) than the controls. In Trial IV (Table XII) the fertility was considerably, but not significantly decreased. Ninety minutes of incubation in

the presence of the homogenate significantly lowered fertility in Trial II (P .01)(Table X).

The UV tissue supernatant had no effect on fertility after 30 minutes incubation (Trial III, Table XI). However, after 60 and 90 minutes, it caused a decreased fertility comparable to the UV homogenate.

The effect of the UV tissue on duration of fertility was parallel to its effect on fertility. In each trial, after 60 minutes of incubation there was a shorter duration of fertility and in Trials I and V the decrease was significant (P .05). In the latter trial, it was not significant from the stirred control (Table XIII). Ninety minutes proved detrimental to fertility as well, and in Trial II differed significantly from the controls (P .01). The UV homogenate supernatant showed similar decreased fertility effects.

5.2.3. Intra-uterine Insemination by Means of Cannulation

Two techniques previously described to assure insemination past the UV junction were utilized. In the first method, the bird laid a hard-shelled egg (the first egg was extremely small weighing 20 gm) on the day of the operation, and continued laying regularly. This hen was inseminated

twice; the first time 3 days post-surgery resulted in 91.7% fertility (of 12 eggs), and the second insemination 24 days post-surgery resulted in 92.3% fertility. The duration of fertility was 17 and 19 days, respectively.

Of the 5 hens treated by the second method, 2 birds laid hard-shelled eggs 8 and 22 days after the operation (#244 and 206). The latter's cannula was removed by the hen 74 days post-surgery.

One bird (#291) laid a shell-less egg 41 days and a hard-shelled egg 47 days after the operation. This bird continued to lay both types of eggs with the majority being shell-less. The fourth bird (#208) laid a shell-less egg after 28 days and subsequently oviposited only shell-less eggs, while the fifth (#256) did not resume laying after surgery.

Birds #244 and 206 were inseminated (Figure 10)following the initiation of egg production. These hens had a mean fertility of 90% and duration of 15.8 days (Table XIV).

5.3. Inter- and Intra-Specific Heterospermic Inseminations and Sperm Competition

5.3.1. Competition Between High and Low Fertility Birds

The .05 mg level of F.I.T.C. was used in marking

sperm to compare sperm transport in high (RIR x SCWL) and low (RIR) fertility males within a single female. In 30 minutes post-insemination, significantly more RIR sperm were recovered in the vaginal area in the 2 birds with mixed semen ($P < .05$). The sperm numbers of the 2 females were not significantly different in the U V area with 54.5% of the sperm from the RIR x SCWL males. After 60 minutes, 1 hen had more RIR sperm in the vaginal area ($P < .1$). No difference was noted in the U V area with 53.3% of the sperm being from the RIR x SCWL males. Both birds tested after 90 minutes showed sperm in the vagina favouring the RIR males (1 $P < .05$ and 1 $P < .01$). The sperm recovered in the U V junction area resulted with one of them having more RIR x SCWL sperm ($P < .1$) and the RIR x SCWL males had 59.8% of the sperm in the 2 females combined.

The numbers of sperm observed in the infundibulum in all birds were very small, and, as a result, could not be analyzed (Table XV).

5.3.2. Inter-species Inseminations in Chickens Following Surgical Removal of the UV Junction

None of 140 eggs and 2 of 29 (6.9%) collected from intact hens were fertile following inseminations with turkey and pheasant semen, respectively. Neither of the 2 fertile

eggs hatched. Two of 53 eggs (3.7%) and 8 of 56 (14.3%) eggs collected from UV junction-less hens were fertile when inseminated with turkey and pheasant semen, respectively.

In observing sperm transport of these two species within the chicken, it was noticed that both are capable of reaching the anterior area of the tract. When turkey sperm were in competition with rooster sperm within a single female, significantly more turkey sperm was found in the vagina after 30 and 60 minutes of intra-vaginal inseminations, while after 90 minutes the majority of sperm recovered were from chicken males. Chicken males had a higher number of sperm at the UV junction ($P < .05$) after 90 minutes but no differences were observed 30 and 60 minutes post-inseminations (Table XVI).

5.4. Competition Between RIR and SCWL Males

Initial inseminations (Aug. 10, 1970) using RIR males in MMI females resulted in 15.9% of the eggs being fertile while SCWL showed 56.3% fertility in the same breed of females. Following heterospermic inseminations, 23 progeny were hatched all of which were sired by the SCWL males. When semen from RIR males was inseminated into MMI females, which had been selected for high fertility, 44.2% of the

eggs incubated were fertile. However, 13 chicks which hatched after mixed inseminations (RIR and SCWL) in the same trial were all sired by the SCWL males. These trials led to the competitive evaluations of the RIR and SCWL breeds.

In the week of November 2, 1970, the concentration of sperm between the 2 breeds of males (5.310×10^9 sperm/ml for SCWL and 5.250×10^9 sperm/ml for RIR) was very similar. However, by the third week the SCWL males had a decided advantage (4.995×10^9 vs 4.178×10^9 sperm cells/ml) and this was maintained until February 22, 1971 (Appendix Table II).

Semen volume greatly favoured the RIR males, which ranged from .391-.598 ml/male with the SCWL ranging from .075-.259 ml/male. The RIR x SCWL males, a group of known high fertility, were included periodically and in all cases had a higher sperm concentration than the other types of males and a volume which was approximately mid-way between the two parental types.

Subjective observations of the semen samples failed to show any differences in sperm motility or rate. There was a slightly higher percentage of sperm with cytoplasmic droplets noted in the RIR semen.

Heterospermic inseminations involving these breeds of males in MMI females resulted in a deficiency of chicks

sired by RIR males in 3 of 4 trials. In the fourth trial, exactly half of the 128 chicks were sired by each. By combining the 4 trials, and expecting equal number of offspring sired from each breed, there was a significantly larger number of white chicks ($P < .01$)(Table XVII).

However, this does not take the fertility of the 2 breeds into account. Throughout the trial period, eggs from females inseminated with RIR males were fertile 42.3% of the time and from the SCWL 57.8% of the time. Thus, one might expect 132.81 of the 230 chicks to be sired by the SCWL, rather than 115 if only sperm numbers were considered,when using the calculations described in the method section. Using this method, no deviation from the expected was found in the total of the 4 trials or in any of the individual trials. A further possibility for differences in ratios may be created by more unrecognizable early deads in chicks sired by one of the breeds. When tested in this manner none of the differences showed significance. The correlation between the percentage of white chicks expected based on the fertility of the 2 breeds (as calculated by the formula presented previously) and the actual per cent of white chicks was found to be .8489.

The superiority of the SCWL males was more predominant when using the RIR females. In the first three trials, RIR

sperm cells and SCWL sperm cells were inseminated in the ratios of 1:1 and 2:1. In the 1:1 ratios, 92% of the offspring were white and in the 2:1 ratio, 79.1% were white. These deviations were highly significant from the expected in all cases. When fertility and recognizable offspring were considered in the 1:1 ratio, the estimation of the expected offspring was much more accurate but were still significantly different from the expected.

The ratio of sperm was then increased to 2.5:1, 3:1, and 4:1 (RIR:SCWL) in an effort to equalize the number of progeny produced by each breed. This resulted in 71.4, 70.8 and 69.4% of the chicks being white, respectively (Table XVIII).

To give a clearer assessment of the fertility of RIR males mated to RIR females, both breeds were inseminated in SCWL females to see if there was an effect due to closely related females. Here the RIR males resulted in 32.9% fertility and the SCWL in 55.6%. Finally, both males were used to inseminate RIR x SCWL females. These were equally related to both except for the Z chromosome. The RIR showed 34.7% and the SCWL 54.2% fertility. In these two trials the fertility was not different from the percentage described in either the MMI or RIR females.

Experiments were then designed to determine competitive

sperm transport between the two breeds within a single RIR x SCWL to see if the ratio of offspring could be explained by differences in sperm numbers at different sites within the tract. No differences were noted in the number of sperm located in the vagina 30 minutes after intra-vaginal heterospermic inseminations. After 60 ($P < .05$) and 90 ($P < .01$) minutes, more RIR sperm were recovered from this area. The SCWL sperm reached the U V area in greater numbers ($P < .1$) in 60 (62.7%) and 90 (61.4%) minutes. No analysis was considered in areas of the tract anterior to the UV junction since small numbers of sperm were recovered. In the various parts of the tract above the UV junction, the SCWL showed a greater number of sperm cells in comparison to the RIR in 67% of the observations (Table XIX).

In order to avoid the barrier mechanism of the UV junction and in an attempt to obtain equal numbers of sperm at the site of fertilization, 12 MMI females were inseminated via cannulations as described in section 4.4.3., method I. Unfortunately, all birds laid only shell-less eggs following the operation.

Following surgical intra-magnal inseminations of the 9 birds inseminated with RIR semen, 2 laid eggs within 14 days of the operation. From eight eggs laid, fertility was 100%. These 2 birds along with 2 others laid fertile eggs

with a mean duration of 23.5 days (range 18-34). None of the hens inseminated with SCWL laid an egg within the 14-day period; however, 3 hens laid fertile eggs after this date. Duration in these hens averaged 22.3 days (range 20-25).

The trial in which the RIR females were inseminated with RIR semen 4 days after being inseminated with SCWL semen resulted in approximately equal numbers of offspring from days 2-15 post-insemination, following the second insemination. Forty-one point seven per cent of the offspring were sired by the RIR males on day 2 and 46.2% in the 14-day total. Both figures were not significantly different from an expected value of 50%. In the reciprocal cross, the SCWL sired 54.5% of the chicks in day 2 and sired 83.9% of the progeny in the 14 days; this figure was highly significant ($P < .001$). The RIR were less successful in SCBrL females in that they sired 12.5% (1 of 8) of the offspring when their semen was used after the SCWL and 7.7% (1 of 13) when used before.

DISCUSSION

F.I.T.C., at a concentration of .05 mg/2.00 x 10^9 sperm/ml, proved to be effective in labelling chicken sperm cells without reducing their fertilizing ability. This is in agreement with the work reported by Mellish (1969) using this fluorochrome with boar sperm. It allowed for comparisons of sperm competition between 2 breeds or species within a single female and thus helped to elucidate differences in offspring ratios following heterospermic inseminations. As well, the stained sperm made possible the rapid scanning of slides, as compared to conventional methods, in determining sperm counts following recovery from various parts of the reproductive tract.

Despite the many advantages offered by this fluorochrome, there are some drawbacks. The tedious and lengthy work of recovering and counting sperm was not eliminated. As well, these sperm could not be identified in histological studies. For this technique to be more useful, a better method of sperm recovery is required in areas anterior to the UV junction.

The gentle stirring of the sperm with the F.I.T.C. to obtain a homogenous distribution of the dye seemed to

serve an added purpose. All the stirred samples showed a consistent and, at times, significantly higher fertility than the non-stirred control. Both Van Wambeke (1968) and Proudfoot and Stewart (1967) have reported that oxygen can have a beneficial effect on fertility. The stirring caused a greater number of sperm to come in contact with the atmosphere, possibly explaining the fertility differences.

Lake and Gilbert (1962) and Gilbert (1969) noted that surgical thread placed in the uterus causes the oviposition of shell-less or soft-shelled eggs. These birds showed fewer sperm in the UV storage area than the controls (Gilbert et al., 1968b). Following the surgical removal of the UV junction (section 4.4.1.), 1 bird in trial II and 5 birds in trial III continually laid shell-less eggs, while all the other birds laid shell-less or soft-shelled eggs intermittently. All 12 MMI females laid only shell-less eggs after cannulation of the uterus (section 4.5.3.). It should be noted that day-time temperature ranged from 85-95^{o}F at this time and soft-shelled and shell-less eggs were common among the non-operated MMI females. The operated MMI birds did resume production of hard-shelled eggs following the annual moult (2 to 3 months post-surgery). Three of these hens retained their cannulae; however, these were blocked and sperm were not able to pass through the tube.

The work has shown that oviposition is possible with a surgical disruption of the tract and that oviposition is not the criterion for ovulation since a number of ova were found in the tract at one time. As well, the tract is capable of contractions in both directions, as has been suggested by Parker (1931), since hard-shelled eggs were found in the abdominal cavity after presumably moving anteriorly in the tract following shell deposition.

The results would indicate that the infundibular area is capable of maintaining prolonged sperm storage and fertility, or that sufficient tubules remained in the uterovaginal area to provide for sperm storage. This is in agreement with Schindler et al. (1967) who reported that these glands can maintain fertility at least as long as the UV tubules, and with Takeda (1966) and Van Krey et al. (1966), who found sperm almost exclusively in the infundibular glands following deep inseminations.

Gilbert et al. (1968a) reported finding transitional and uterine glands anterior to the UV junction tissue and Fujii and Tamura (1963) observed sperm tubules in the "beginning" of the vaginal area that were capable of storing sperm. It is possible that the tubules other than the ones found in the UV junction were seen in the present work. The area from which the tubules originated could not be determined

in our laboratory. The birds generally did show an increased fertility with a longer duration between surgery and insemination. Possibly a regeneration of the removed tissue occurred or the uterine and vaginal tubules replaced the UV sperm host glands as the main sperm storage area.

Grigg and Skaller (1958) found three sterile hens when inseminated by conventional methods. However, with intraperitoneal inseminations, the birds laid 90% fertile eggs. They postulated that the initial failure of fertility was due to "an exaggeration of the restrictive function of the utero-vaginal junction." In the present study, the females inseminated anterior to the UV junction via cannulae all showed fertility in excess of 90% with a mean duration of 16.5 days.

There is some thought that following inseminations which avoid the UV barrier, there would be a greater possibility of fertilization being accomplished by genetically inferior or foreign sperm. As can be seen from Table VIII, only 41 out of 87 fertile eggs from UV junctionless birds hatched in trials IV and V combined. The high embryonic mortality (52.9%) agrees with the findings of Ogasawara et al. (1966) and Van Krey et al. (1966) who found an increase in embryonic deaths with inseminations past the UV junction. Intra-magnal inseminations in chickens with turkey semen increased fertility when compared to conventional inseminations

(Kempenich-Pinto et al., 1970) and in these hens nearly all the sperm were located in the infundíbulár glands (Schindler et al., 1970). Intra-vaginal inseminations of intact chickens with turkey and pheasant semen in the present report resulted in 0 and 6.9% fertility, respectively. In hens where the UV barrier was removed, fertility was increased to 3.7 and 14.3%. It would appear that the UV junction decreases the number of sperm, both inter- and intra-specific, from reaching the site of fertilization.

It has been well established that avian sperm can remain viable in the female tract for a long duration (Crew, 1926). However, sperm incubated with UV junction homogenate show a higher metabolic rate (Van Krey et al., 1970) and Dukelow and Williams (1967) have indicated that an increased respiratory rate decreases sperm's viability. The work in which sperm were incubated with the UV tissue agree with Dukelow and Williams' suggestion regarding decreased fertility. It should be noted that non-incubated sperm accompanied with UV homogenate caused a consistent but non-significant reduction in fertility. It appears that there could have existed some other agent causing the lowered fertility. Bacterial contamination and unequal sperm numbers per insemination certainly remain as possibilities.

Warren and Kilpatrick (1929)(using chickens) and Payne

and Kahrs (1961)(using turkeys) have reported that new males sire practically all the offspring in a short time after replacing the old ones. This was confirmed in section 5.3.1. when SCWL semen followed the RIR semen; however, the reciprocal cross proved contrary to these reports. It should be noted that Payne and Kahrs (1961) observed that fresh semen from turkey males non-related to the females were more effective in replacing sperm present from previous males than was fresh sperm from males closely-related to the females. This could possibly explain the RIR male performance in the RIR females.

Workers observing sperm competition following heterospermic inseminations found significant deviations from the expected offspring ratio in chickens (Buckland and Hawes, 1968), rabbits (Beatty, 1960), cows (Beatty et al., 1969), and pigs (Mellish, 1969). These workers only considered sperm numbers in their analyses and neglected to consider other parameters such as fertility of the males and the number of sperm reaching the site of fertilization from each of the males.

The research involving heterospermic inseminations has illustrated that it is necessary to look beyond differences in sperm numbers between the sires in calculating the ratios of expected offspring. When semen from RIR and SCWL males was inseminated into MMI females, the fertility of each of the breeds was utilized to calculate the percentage of

white offspring expected from each mixed insemination. A correlation of .8489 was calculated between the percentage of white chicks expected (based on the combined fertility) and the actual percentage of white chicks obtained in the 4 trials.

With the substitution of RIR females for the MMI's, the fertility percentages of the two breeds were not sufficient in estimating the expected percentage of white offspring. However, in considering the fertility percentages, the derived expected was more accurate than the usual 1:1 expected. With these females, the ratio of offspring was not altered significantly when the ratio of sperm ranged from 2.5:1 to 4:1 in favor of the RIR males. Here it was probably more important to consider the number of sperm from each breed reaching the site of fertilization. In the studies conducted, the SCWL males had significantly more sperm at the UV junction 60 and 90 minutes after inseminating equal number of sperm from each breed. At these times there were significantly more RIR sperm in the vagina. It would have added to the information collected to test the number of sperm from each breed reaching the UV junction in connection with changes in the ratio of sperm cells from the different males as discussed above. Perhaps the number of sperm at the UV junction from each male did not change

despite the shifts in sperm ratios inseminated.

Dunn (1927) and Bhatnagar (1963) reported better fertility when females were inseminated with closely related males. Allen and Champion (1955) along with this work could not substantiate these findings. No differences within male fertility were noted in any of the 5 breeds of females inseminated. In each trial, the SCWL males showed higher fertility. When the RIR sperm were in competition with the SCWL sperm following heterospermic insemination, the RIR males fared better in the MMI females than in the RIR females.

The inferior performance of the RIR males cannot readily be explained. RIR sperm were capable of remaining viable in the female tract for relatively long periods as shown by the mean duration of 23.5 days (1 female showed fertility 34 days post-insemination) following surgical intra-magnal inseminations. It would seem that the UV junction prevented a prolonged fertility when using RIR sperm.

From the above studies, it can be concluded that the function of the UV junction could be considered similar to that of the cervix in the cow and utero-tubal junction in the pig, i.e., decreasing the number of sperm ascending the tract. It may act as a "filtering agent" by limiting the number of sperm reaching the anterior portion of the tract

and participating in fertilization.

SUMMARY AND CONCLUSIONS

The studies conducted in this report clarified some aspects of the chicken's oviduct function in respect to intra- and inter-species sperm transport. A suitable irreversible sperm marker helped in these studies to determine sperm competition and to count sperm rapidly. Experiments were designed to evaluate the importance of the UV junction by removing this tissue and by observing the effects of inseminating sperm both posterior and anterior to this area. From the work it can be concluded that:

1) F.I.T.C., at a level of .05 mg/2.00 x 10^9 sperm cells/ml can stain sperm without affecting their fertilizing ability;
2) the UV junction is not necessary for prolonged fertility and acts by limiting the number of sperm ascending the tract;
3) high fertility birds (SCWL) sired more offspring than low fertility birds (RIR) in heterospermic inseminations - in the experiment involving MMI females, the discrepancies in the ratio of offspring was explained by the difference in fertility of the 2 male breeds;
4) fertility involving inter-specific inseminations increased

in UV junction-less hens indicating the barrier effect of this sphincter;

5) competition between sperm of low and high fecundity males inseminated into a single female resulted in the sperm from the high fertile males reaching the UV junction first; similarly, rooster sperm arrived at the UV junction prior to turkey semen following heterospermic insemination of the 2 species within a single female.

Table I. The use of acridine orange and F.I.T.C. to study their influences as a sperm marker

Time (Post A.I.)		Oviduct Sections Examined		
		Vagina	UV Junction	Infundibulum
Acridine Orange[2]				
30 Min.	No. Observed	159	7	
	No. Estimated	54,900	211	
	% Stained	0	0	
60 Min.	No. Observed	116	6	
	No. Estimated	39,400	175	
	% Stained	0	0	
90 Min.	No. Observed	149	16	
	No. Estimated	50,700	472	
	% Stained	0	0	
F.I.T.C.[2]				
30 Min.	No. Observed ±S.E.[1]	136±5.206	6±2.510	1.7±0.882
	No. Estimated	64,233	69.7	3.7
	% Stained	100	100	100
60 Min.	No. Observed ±S.E.[1]	142±27.610	10.7±6.667	9.7±5.239
	No. Estimated	48,277	156	117.3
	% Stained	100	100	100
90 Min.	No. Observed ±S.E.[1]	131±18.520	44±17.534	2.0±1.155
	No. Estimated	37,644	592	6.7
	% Stained	100	100	100

[1] Numbers based on a mean of 3 birds.

[2] .05 mg of each fluorochrome per ml of semen (2.00×10^9 sperm cells).

Table II. The effect of different levels of F.I.T.C.[1] on fertility in the hen (Trial I)

Semen Treatment	No.of Birds Inseminated	No.of Eggs	% Production	% Fertile
Immediate A.I.	20	208	74.3	64.4
Control I (non-stirred)	20	211	75.4	49.3
Control II (stirred)	20	229	81.8	65.1
F.I.T.C.-.025	21(20)[2]	224(220)	78.9(78.6)	60.0(60.0)
F.I.T.C.-.050	20	224	80.0	60.3
F.I.T.C.-.100	20	207	73.9	46.9

[1]Expressed as ml of .001 solution of F.I.T.C. per ml of semen (2.00×10^9 sperm cells).

[2]Values in brackets were those used for statistical analysis.

Analysis of Variance for Percentage Fertility

Source	d.f.	SS	MS	F.calc.
Total	119	6.9227		
Treatments	5	.6184	.1237	2.2363[a]
Error	114	6.3043	.0553	

[a]Significant at .1 level (2.29 needed for .05 level).

Table III. The effect of different levels of F.I.T.C.[1] on fertility in the hen (Trial II)

Semen Treatment	No.of Birds Inseminated	No. of Eggs	% Production	% Fertile		
Immediate A.I.	19	182	68.4	74.7	a	a
Control I (non-stirred)	20(19)[2]	197(191)	70.4(71.8)	52.3(51.8)	a	b
Control II (stirred)	21(19)	200(190)	68.0(71.4)	54.0(53.7)	a	b
F.I.T.C.-.025	19(19)	195	73.3	56.9	a	ab
F.I.T.C.-.050	20(19)	207(201)	73.9(75.6)	68.6(68.1)	a	ab
F.I.T.C.-.100	19(19)	186	69.9	19.9	b	c
					.01	.05

[1]Expressed as ml of .001 solution of F.I.T.C. per ml of semen (2.00×10^9 sperm cells).

[2]Values in brackets were those used for statistical analysis.

Figures in rows having the same superscripts are not significantly different from one another at the level indicated.

Analysis of Variance for Percentage Fertility

Source	d.f.	SS	MS	F.calc.
Total	113	13.6590		
Treatments	5	3.3201	.6640	6.9362**
Error	108	10.3390	.0957	

**$p < .01$

Table IV. The effect of different levels of F.I.T.C.[1] on fertility in the hen (Trial III)

Semen Treatment	No.of Birds Inseminated	No. of Eggs	% Production	% Fertile
Immediate A.I.	18(14)[2]	174(145)	60.9(74.0)	58.0(58.6)
Control I (non-stirred)	14	124	63.3	45.2
Control II (stirred)	15(14)	146(138)	69.5(70.4)	55.5(55.1)
F.I.T.C.-.025	14	134	68.4	53.0
F.I.T.C.-.050	15(14)	133(128)	63.3(65.3)	55.6(57.8)

[1]Expressed as ml of .001 solution of F.I.T.C. per ml of semen (2.00×10^9 sperm cells).

[2]Values in brackets are those used for statistical analysis.

Analysis of Variance for Percentage Fertility

Source	d.f.	SS	MS	F.calc.
Total	69	5.3798		
Treatment	4	.2215	.0554	.6977
Error	65	5.1583	.0794	

Table V. Time required for initiation or resumption of egg production following the removal of the UV junction in the 3 trials.

Time required for production (days post-surgery)	Trial I (No. females)	Trial II (No. females)	Trial III (No. females)
0-14	-	-	8
15-28	-	3	7
29-42	-	1	3
43-98	2	-	2
98	2	1	1
Total	4	5[1]	21[2]
% of total birds operated	33.3	55.5	70.0

[1]One female laid only shell-less eggs.

[2]Four females laid only shell-less eggs.

Table VI. The effect of oviduct surgery on subsequent egg production and fertility (Trial II)

Insemination Sequence	Bird Number	Time of A.I. (days post-surgery)	Eggs Laid (days 2-15 post A.I.)	Eggs Fertile (%)	Duration of Fertility (days)
1st	3384 (sham)	25	5	0	-
	1532	24	4	0	-
	3355	26	5	0	-
	3361	38	7	100.0	14
	Means	28.2	5.1	33.3	3.5
2nd	3384 (sham)	39	4	75.0	12
	1532	40	0	-	-
	3355	39	8	75.0	14
	3361	103	0	-	-
	Means	55.2	3.0	75.0	6.5
3rd	3384 (sham)	104	0	-	-
	1532	75	2	100.0	4
	3355	74	6	100.0	13
	Means	84.3	2.7	100.0	5.7
4th	1532	105	0	-	-
	3355	104	7	100.0	13
	Means	104.5	3.5	100.0	6.5

Table VII. The effect of oviduct surgery on subsequent egg production and fertility (Trial III)

Insemination Sequence	No. Birds	Days Post-Surgery ($\bar{X}$)	No. Eggs (Days 2-15 post A.I.)	Egg Production (%)	Fertility (%)
1st	5	17.2	15	21.4	33.3
2nd	6	39.7	42	50.0	35.7
3rd	6	53.7	36	42.9	27.8
4th	8	84.0	60	53.6	85.0
5th	5	129.4	44	62.9	81.8
Control I[1]	16	-	157	70.1	65.0
Control II[2]	16	-	153	68.3	71.0

[1]Control I was run concurrently with insemination #4.

[2]Control II was run concurrently with insemination #5.

Table VIII. Hatchability of fertile eggs from the birds in which the UV junctions were removed in Trials IV and V

Trial	Days Post A.I.	No. Birds	No. Eggs	No. Fertile (%)	No. Hatched (%)	No. Pips (%)	No. Dead (%)
IV	2-8	8	34	34 (100)	17 (50)	4 (11.8)	13 (38.2)
	9-15	8	26	17 (65.4)	5 (29.4)	4 (23.5)	8 (47.1)
	Total	8	60	51 (85.0)	22 (43.1)	8 (15.7)	21 (41.2)
V	2-8	7	21	19 (90.5)	11 (57.9)	3 (15.8)	5 (26.3)
	9-15	7	23	17 (73.9)	8 (47.1)	4 (23.5)	5 (29.4)
	Total	7	44	36 (81.8)	19 (52.7)	7 (19.4)	10 (27.8)

Table IX. The effect of sperm incubated with UV junction homogenate on fertility. (Trial I)

Treatment	Time[1] (min)	Birds inseminated (NO.)	Eggs incubated (NO.)	Fertility (%)		Duration Days ($\bar{X}$) ± S.E.	
Control	0	12(10)[2]	132(111)	87.9(87.0)	a a	14.6(14.8)±0.814	ab
Control	60	10	113	79.7	a ab	15.3 ±0.764	a
Buffer[3]	60	11(10)	122(115)	63.9(64.3)	b b	12.1(12.3)±1.126	bc
UV Homogenate[4]	60	10	101	35.6	c c	9.4 ±1.714	c
					.05 .01		.05 & .01

[1]Time in minutes between collection of semen and insemination.
[2]Value in brackets refers to values actually used for statistical analysis for percentage and duration of fertility.
[3]Buffer added at a ratio of 1:2.
[4]The ratio of UV homogenate added to semen was 1:2.

Figures in rows having the same superscripts are not significantly different from one another at the level indicated.

Analysis of Variance

Source		d.f.	S.S.	M.S.	F. calc.
Fertility					
	Total	9	2.74646698		
	Treatment	3	1.67793728	.55931242	20.205**
	Error	36	1.06852970	.02968138	
Duration					
	Total	39	702.9		
	Treatment	3	219.7	73.23333·	5.4561**
	Error	36	483.2	13.42222·	

** P < .01

Table X. The effect of sperm incubated with UV junction homogenate on fertility. (Trial II)

Treatment	Time[1] (min)	Birds inseminated (no.)	Eggs incubated (No.)	Fertility (%)		Duration Days ($\bar{X}$) ± S.E.	
Control	0	10	105	86.7 a	a	15.6 ±0.933	a
Control	90	13(10)[2]	141(116)	54.1(53.4) bc	a	11.0(11.4)±2.301	a
Buffer[3]	90	12(10)	131(115)	64.9(66.1) ab	a	12.9(12.6)±2.291	a
UV Homogenate[4]	90	14(10)	145(117)	15.2(11.1) d	b	3.4(2.7)±1.001	b
				.05 .01			.05 & .01

[1]Time in minutes between collection of semen and insemination.
[2]Value in brackets refers to values actually used for statistical analysis for percentage and duration of fertility.
[3]Buffer added at a ratio of 1:2.
[4]The ratio of UV homogenate added to semen was 1:2.

Analysis of Variance

Source		d.f.	S.S.	M.S.	F. calc.
Fertility					
	Total	39	6.43987438		
	Treatment	3	3.16329168	1.05443056	11.5851**
	Error	36	3.2765827	.09101618	
Duration					
	Total	39	2037.775		
	Treatment	3	920.475	306.8250	9.8861**
	Error	36	1117.300	31.0361	

**P < .01

Table XI. The effect of sperm incubated with UV junction homogenate on fertility. (Trial III)

Treatment	Time[1] (min)	Birds inseminated (No.)	Eggs incubated (No.)	Fertility (%)	Duration Days ($\bar{X}$) ± S.E.
Control	30	9	104	82.7	14.6 ±1.237
Control	45	10(9)[2]	116(107)	83.6(82.2)	14.8(14.6)±0.915
Buffer[3]	45	11(9)	134(112)	88.1(86.6)	15.1(14.6)±1.879
UV Homogenate[4]	45	11(9)	123(108)	89.4(88.0)	15.2(15.4)±1.015

[1]Time in minutes between collection of semen and insemination.
[2]Value in brackets refers to values actually used for statistical analysis for percentage and duration of fertility.
[3]Buffer added at a ratio of 1:2.
[4]The ratio of UV homogenate added to semen was 1:2.

Analysis of Variance

Source		d.f.	S.S.	M.S.	F. calc.
Fertility					
	Total	35	1.10507956		
	Treatment	3	.04816089	.01605363	0.4861
	Error	32	1.05691467	.03302858	
Duration					
	Total	35	504.3333·		
	Treatment	3	5.3333·	1.7777·	0.1140
	Error	32	499.0	15.593375	

Table XII. The effect of sperm incubated with UV junction homogenate on fertility. (Trial IV)

	Treatment	Birds inseminated (No.)	Eggs incubated (No.)	Fertility (%)	Duration Days ($\bar{X}$) ± S.E.
No Incubation					
	Control	12(11)[1]	117(112)	65.0(66.1)	10.8(11.3)±1.287
	Buffer[2]	12(11)	128(120)	60.9(60.8)	10.1(10.1)±1.729
	Supernatant[3]	11	110	58.2	9.8 ±0.412
	UV Homogenate[4]	11	118	44.1	7.5 ±1.282
60 Min.of Incubation					
	Control	9(8)	88(82)	62.5(63.4)	10.0(9.9)±1.918
	Buffer	8	87	47.1	10.9 ±2.000
	Supernatant	8	82	31.7	6.5 ±1.956
	UV Homogenate	8	80	28.8	6.4 ±1.818
90 Min.of Incubation					
	Control	8(7)	84(75)	60.7(65.3)	10.9(11.4)±2.057
	Buffer	8(7)	85(79)	54.1(54.4)	10.9(10.9)±1.969
	Supernatant	7	80	38.8	8.0 ±2.104
	UV Homogenate	8(7)	78(71)	34.6(36.6)	5.6(7.0)±1.732

[1]Value in brackets refers to values actually used for statistical analysis for percentage and duration of fertility.
[2]Buffer added at a ratio of 1:2.
[3]Supernatant added at a ratio of 1:2.
[4]The ratio of UV homogenate added to semen was 1:2.

Analysis of Variance for Trial IV

		Source	d.f.	S.S.	M.S.	F. calc.
No Incubation						
	Fertility	Total	43	4.66180464		
		Treatment	3	0.19216300	.06405433	0.5732
		Error	40	4.46964164	.11174104	
	Duration	Total	43	904.88636364		
		Treatment	3	84.43181818	28.14393939	1.3721
		Error	40	820.45454546	20.51136363	
60 Min.of Incubation						
	Fertility	Total	31	3.49648672		
		Treatment	3	.62477359	.20825786	2.0306
		Error	28	2.87171313	.10256118	
	Duration	Total	31	1015.71875		
		Treatment	3	128.09375	42.6979166	1.3469
		Error	28	887.625	31.7008985	
90 Min.of Incubation						
	Fertility	Total	27	2.49881872		
		Treatment	3	.34490986	.11496995	1.2811
		Error	24	2.15390886	.08974620	
	Duration	Total	27	787.85714286		
		Treatment	3	135.28571428	45.09523809	1.6505
		Error	24	652.57142858	27.19047619	

Table XIII. The effect of sperm incubated with UV junction homogenate on fertility. (Trial V)

	Treatment	Birds inseminated (No.)	Eggs incubated (No.)	Fertility (%)	Duration Days ($\bar{X}$) ± S.E.
No Incubation					
	Control	8	77	64.9	11.8 ±0.996
	Buffer [2]	8	84	66.7	11.1 ±1.274
	Supernatant[3]	8	78	51.3	9.8 ±1.264
	UV Homogenate[4]	8	78	50.0	9.9 ±1.563
30 Min.of Incubation					
	Control	8	84	61.9	11.6 ±0.999
	Buffer	9(8)[1]	86(78)	64.0(65.4)	11.6(11.9) ±1.172
	Supernatant	8	75	49.3	10.1 ±0.934
	UV Homogenate	8	75	42.7	8.4 ±1.085
60 Min.of Incubation					
	Control	8	90	60.0^{a}	10.0 $\pm 1.648^{ab}$
	Buffer	9(8)	90(84)	$58.9(59.5)^{a}$	11.3(11.4) $\pm 0.999^{a}$
	Supernatant	8	73	30.1^{b}	5.9 $\pm 1.432^{b}$
	UV Homogenate	8	85	28.2^{b}	6.1 $\pm 1.481^{b}$

[1]Value in brackets refers to values actually used for statistical analysis for percentage and duration of fertility.
[2]Buffer added at a ratio of 1:2.
[3]Supernatant added at a ratio of 1:2.
[4]The ratio of UV homogenate added to semen was 1:2.

Figures in rows having the same superscripts are not significantly different from one another at the level indicated.

Analysis of Variance for Trial V

		Source	d.f.	S.S.	M.S.	F. calc.
No incubation						
	Fertility	Total	31	2.04785372		
		Treatment	3	.16381864	.05460628	0.8115
		Error	28	1.88403488	.06728692	
	Duration	Total	31	395.5		
		Treatment	3	22.75	7.58333·	0.5778
		Error	28	372.75	13.125	
30 Min.of Incubation						
	Fertility	Total	31	1.38965122		
		Treatment	3	.25274034	.08424678	2.0748
		Error	28	1.13691088	.04060396	
	Duration	Total	31	310		
		Treatment	3	62.5	20.83333·	2.356·
		Error	28	247.5	8.83928571	
60 Min.of Incubation						
	Fertility	Total	31	2.2889395		
		Treatment	3	.72284375	2.4094791	4.3079*
		Error	28	1.56609575	.5593199	
	Duration	Total	31	629.21875		
		Treatment	3	183.59375	61.1979166	3.8453*
		Error	28	445.625	15.91517857	

Table XIV. Fertility following intra-uterine inseminations utilizing the cannulation methods

	Insemination No.	Bird No.	Days Post Surgery	No. Eggs	% Fertile	Duration (in days)
Method I	1	505	3	12	91.7	17
	2	505	24	13	92.3	19
	Means		13.5	12.5	92.0	18
Method II	1	206	32	4	100	13
	2	206	47	7	85.7	16
	3	244	61	10	90	18
	4	206	68	9	88.9	16
	Means		52	7.5	90	15.8

Table XV. Competition of sperm transport between low and high fecundity cocks - RIR vs RIR x SCWL (X)

Time Post A.I.	Sperm Mixture	Vagina[1] No. Observ.	Vagina[1] No. Est'd	Vagina[1] % Stained	UV Junction[1] No. Observ.	UV Junction[1] No. Est'd	UV Junction[1] % Stained	Infundibulum[1] No. Observ.	Infundibulum[1] No. Est'd	Infundibulum[1] % Stained
30	RIRxRIR	141.0±23.941	47,628	57.3	3.3±1.764	99	60.0	3±1.528	9	66.7
Min.	X[a] x X	166.7±18.765	56,672	46.2[b]	5.0±1.881	148	53.3	-		
	RIRx X[a]	81.7±15.412	27,722	55.1	3.3±1.546	36	40.0	1.7±0.745	16	43.8
	RIRx X[a]	135.0±21.818	45,834	42.7**	4.0±1.053	125	50.0	3±1.732	9	55.6
60	RIR[a]xRIR	192.7±31.005	65,483	48.8	7.0±2.309	171	42.8	0.7±0.471	2	33.3
Min.	X[a] x X	65.3±17.117	22,152	55.1	8.0±1.943	227	54.2	1±0.000	6	66.7
	RIR[a]xX	99.7± 8.173	33,852	54.8[b]	12.0±2.411	350	50.0	-	-	-
	RIRxX[a]	47.0±12.051	15,912	52.5	8.0±1.650	240	58.3	1.7±0.882	10	60.0
90	RIR[a]xRIR	77.7±13.135	26,380	43.8[b]	10.0±2.532	297	60.0	1±0.000	6	33.3
Min.	X[a] x X	123.7±11.814	42,012	47.4	8.3±1.291	251	44.0	1±0.577	6	33.3
	RIR[a]xX	110.7±10.949	37,644	59.9***	18.7±3.110	560	44.6	1±0.577	3	33.3
	RIRxX[a]	152.0±14.332	51,700	45.0*	12.7±1.843	380	65.7[b]	-	-	-

[1]Based on a mean of 3 birds.
[a]Denotes stained sperm.
[b]$P < .1$

*$P < .05$
**$P < .01$
***$P < .001$

Table XVI. The use of F.I.T.C. stained sperm to evaluate turkey vs rooster sperm competition within a single female

	30 Min.			60 Min.			90 Min.		
	No. Observ.[1]	No. Est'd	% Rooster Sperm	No. Observ.[1]	No. Est'd	% Rooster Sperm	No. Observ.[1]	No. Est'd	% Rooster Sperm
Vagina	205.7±28.417	69,938	39.7***	183.3±22.667	62,322	46.0**	181.0±17.453	61,540	58.9***
UV	12.3± 2.603	431	54.1	17.0± 2.887	595	46.8	14.3± 3.180	500	67.4**
Uterus	4.3± 2.333	54	38.5	3.3± 1.764	83	40.0	3.0± 1.528	75	33.0
Magnum	2.0± 0.527	25	50.0	1.7± 0.745	21	40.0	2.0± 0.527	25	67.0
Infundibulum	2.0+ 0.667	25	66.7	4.0+ 2.082	50	67.0	3.3+ 1.546	83	30.0

[1]Based on a mean of 3 females.

*P < .05
**P < .01
***P < .001

Chi-Square Analysis

	30 Min.			60 Min.			90 Min.		
	Exp.[1]	Obs.[2]	χ^2	Exp.	Obs.	χ^2	Exp.	Obs.	χ^2
Vagina	308.5	245	26.141***	225	253	6.969**	271.5	320	17.328***
UV	18.5	20	0.243	25.5	24	0.176	21.5	29	5.233**

[1]Expected number of rooster sperm in the 3 females combined.

[2]Actual number of rooster sperm observed in the 3 females.

Table XVII. The ratio of offspring following heterospermic inseminations of RIR and SCWL semen in MMI females

Trial	Males	No. females inseminated	No. Eggs incubated	% fertile	No. chicks observed	White chicks No. (%)	No. White chicks Exp. (based on sperm No.)	χ^2	No. White chicks Exp. (based on % Fertility)	χ^2	No. White chicks Exp. (based on recog.%)	χ^2
I	RIR	13	115	64.3	71	0 (0)	-	-	-	-	-	-
	SCWL	13	128	63.3	63	63 (100)	-	-	-	-	-	-
	Mixture[1]	26	258	58.9	128	64 (50)	64	0	63.5	.008	55.8	2.136
II	RIR	10	104	27.8	21	0 (0)	-	-	-	-	-	-
	SCWL	7	65	58.6	36	36 (100)	-	-	-	-	-	-
	Mixture[1]	16	139	29.7	38	23 (65.8)	19	3.790[a]	25.76	.070	28.03	1.248
III	RIR	7	50	20.0	10	0 (0)	-	-	-	-	-	-
	SCWL	6	49	53.1	23	23 (100)	-	-	-	-	-	-
	Mixture[1]	8	64	73.4	36	29 (80.6)	18	13.444**	25.45	1.690	25.57	1.588
IV	RIR	6	45	46.7	18	0 (0)	-	-	-	-	-	-
	SCWL	9	78	53.8	40	40 (100)	-	-	-	-	-	-
	Mixture[1]	6	46	67.4	28	18 (64.3)	14	2.286	14.98	1.309	15.73	0.748
Total	RIR	36	314	42.3	120	0 (0)	-	-	-	-	-	-
	SCWL	35	320	57.8	162	162 (100)	-	-	-	-	-	-
	Mixture[1]	56	507	53.5	230	136 (59.1)	115	7.780**	132.81	0.181	131.95	0.292

[1]Equal numbers of RIR and SCWL sperm cells.

**P < .01.

[a]P < .1; 3.841 needed for .05.

Table XIX. The use of F.I.T.C. stained sperm to evaluate RIR vs SCWL sperm competition within a RIR x SCWL female

	30 Min.			60 Min.			90 Min.		
	No. Observ.[1]	No. Est'd	% SCWL Sperm	No. Observ.[1]	No. Est'd	% SCWL Sperm	No. Observ.[1]	No. Est'd	% SCWL Sperm
Vagina	244.3±26.171	83,062	47.0	218.0±19.941	74,120	45.9*	168.7±27.354	57,358	40.0**
UV	12.0± 2.309	420	55.6	17.0± 2.887	595	62.7	19.0± 3.215	665	61.4
Uterus	4.0± 2.646	50	50.0	3.3± 1.764	41.3	40.0	4.0± 2.082	50	58.3
Magnum	4.0± 1.732	50	67.0	2.7± 1.453	33.8	62.5	3.3± 1.546	41.3	30.0
Infundibulum	1.7± 0.745	21.3	20.0	6.3± 3.180	78.8	68.4	3.0± 1.732	38.0	67.0

[1]Based on a mean of 3 females.

*P < .05

**P < .01

Chi-Square Analysis

	30 Min.			60 Min.			90 Min.		
	Exp.[1]	Obs.[2]	χ^2	Exp.	Obs.	χ^2	Exp.	Obs.	χ^2
Vagina	366.5	345	2.523	327	300	4.459	253	202	20.561
UV	18.0	20	0.444	25.5	32	3.314	28.5	35	2.965

[1]Expected number of SCWL sperm in the 3 females combined.

[2]Actual number of SCWL sperm observed in the 3 females.

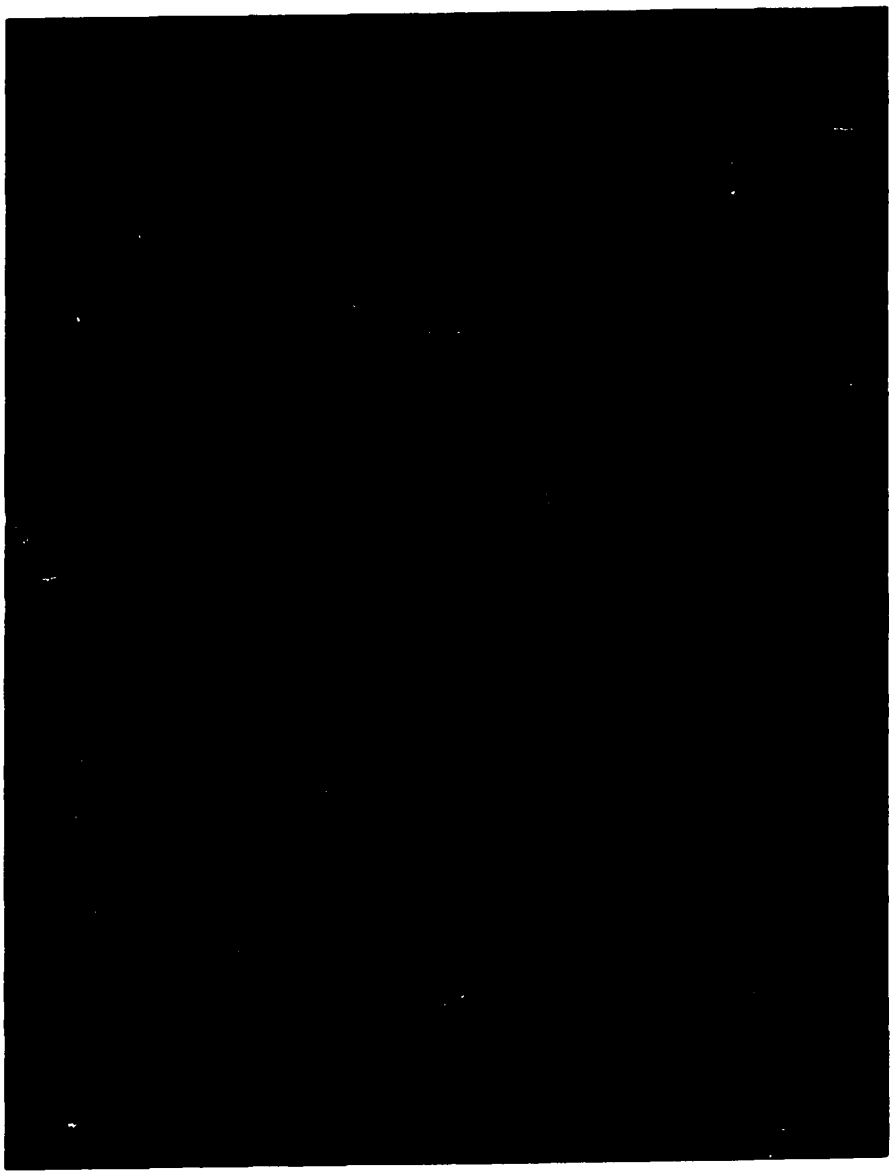

Figure 1. Photomicrograph of rooster spermatozoa, all stained with F.I.T.C., illuminated with ultraviolet light (microscope at 630x when photograph was taken.

Figure 2. The reproductive tract of a normal bird in lay.

Figure 3. The utero-vaginal area of the hen in Figure 2. Note the [illegible] vaginal junction.

Figure 4. The reproductive tract of a bird which had its UV junction surgically removed (#3377)*.

Figure 5. A close-up of the UV junction of hen #3377. This area is well healed and no UV tissue is evident.

*Note the well maintained tract and large follicles although this bird had not laid from operation to time of death (100 days).

Figure 6. A photomicrograph of a typical cross section of tissue removed during the surgical removal of the UV junction area. Note the number of tubules present. (Microscope at 400x when photomicrograph taken).

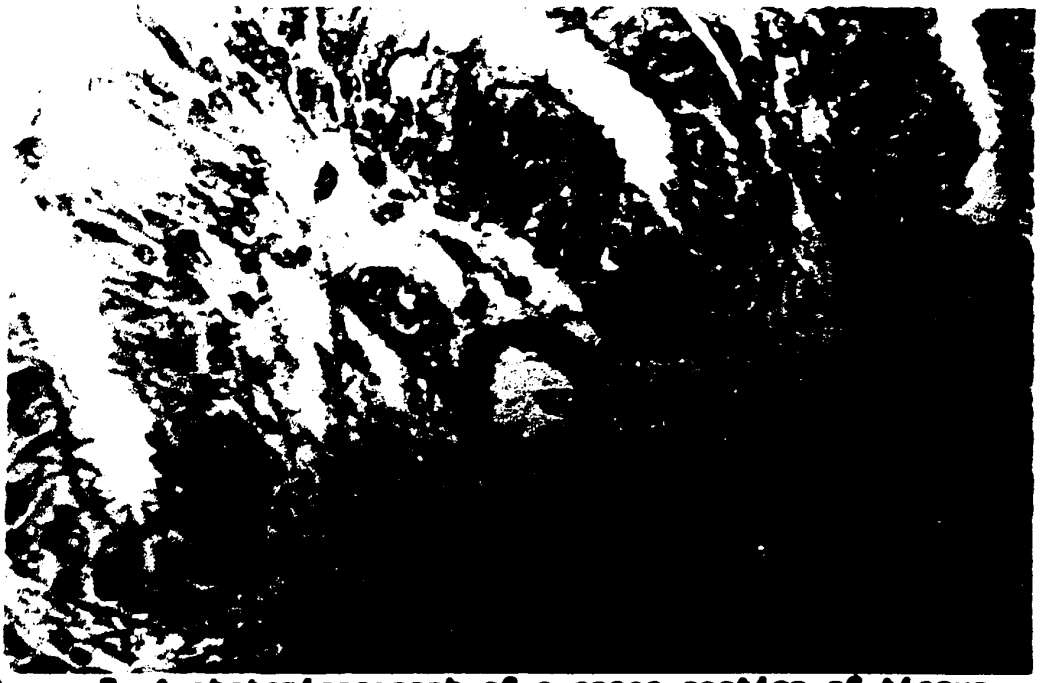

Figure 7. A photomicrograph of a cross section of tissue removed from the area of the vagina and uterus from a UV junction-less hen at the time of sacrifice. Then we had no tubules present in most fields. There appears to be one tubule in the above field. (Microscope at 400x when photomicrograph taken).

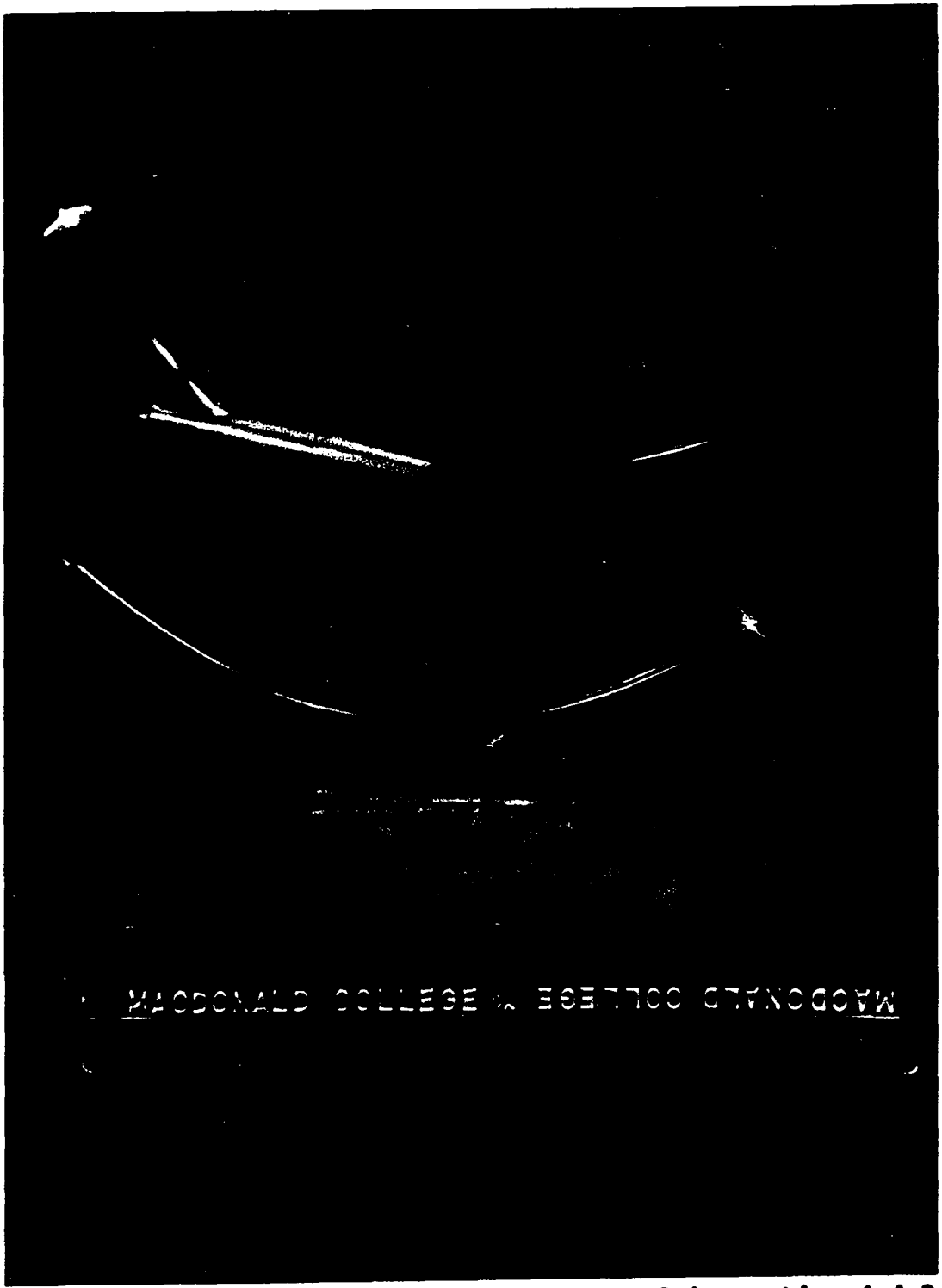

Figure 8. The cannula and hollow tube used in section 4.4.3. Method II. The bevelled end and polyvinyl circle can be noted. There is a demonstration of the cannula sliding out of the slit in the tube.

Figure 9. The trailing end of the cannula in relation to the hen.

Figure 10. Insemination of a hen via the cannula method.

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APPENDIX

Appendix Table I. Initial fertility trials using the RIR and SCWL males and MMI females

Date	Males	No. Hens Inseminated	No. Eggs Incubated	% Fertile	No. Chicks Observed	% White Chicks
Aug.10/70	RIR	1	69	15.9	10	0
	SCWL	6	48	56.3	26	100
	Mix[1]	6	49	49.0	23	100
Sept.8/70	RIR[2]	7	52	44.2	19	0
	Mix[1]	8	54	24.1	13	100

[1]Equal numbers of RIR and SCWL sperm.

[2]The RIR semen was inseminated into females showing high fertility in the Sept. 8/70 trial.

Appendix Table II. Comparisons of semen volume and sperm concentration between RIR and SCWL males. The RIR x SCWL males were included periodically

Date Week Starting	Breed of Roosters	Volume/Male (ml)	Conc. (x 10^9/ml)
Nov. 2, 1970	RIR	.396	5.250
	SCWL	.075	5.310
Nov. 9, 1970	RIR	.546	5.123
	SCWL	.140	5.130
	RIR x SCWL	.263	5.370
Nov. 16, 1970	RIR	.572	4.958
	SCWL	.192	5.0485
	RIR x SCWL	.277	5.258
Nov. 23, 1970	RIR	.589	4.178
	SCWL	.217	4.995
	RIR x SCWL	.283	5.242
Nov. 30, 1970	RIR	.567	4.0875
	SCWL	.213	5.010
	RIR x SCWL	.270	5.318
Dec. 7, 1970	RIR	.539	3.968
	SCWL	.116	4.995
	RIR x SCWL	.250	5.400
Dec. 21, 1970	RIR	.561	3.960
	SCWL	.126	5.025
	RIR x SCWL	.264	
Jan. 4, 1971	RIR	.391	4.275
	SCWL	.143	5.085
	RIR x SCWL	.171	5.225
Jan. 18, 1971	RIR	.598	4.088
	SCWL	.143	5.003
	RIR x SCWL	.347	5.113
Jan. 25, 1971	RIR	.475	4.007
	SCWL	.109	4.775
Feb. 1, 1971	RIR	.587	3.955
	SCWL	.246	4.850

Appendix Table II (cont'd)

Date Week Starting	Breed of Roosters	Volume/Male (ml)	Conc. (x 10^9/ml)
Feb. 8, 1971	RIR	.574	3.850
	SCWL	.213	4.951
	RIR x SCWL	.284	5.225
Feb. 15, 1971	RIR	.559	3.453
	SCWL	.194	4.425
Feb. 22, 1971	RIR	.590	3.775
	SCWL	.179	3.975
Mar. 1, 1971	RIR	.591	3.825
	SCWL	.236	4.125
	RIR x SCWL	.289	4.875
Mar. 15, 1971	RIR	.560	3.545
	SCWL	.259	3.666
Mar. 22, 1971	RIR	.535	3.805
	SCWL	.186	4.225
Mar. 29, 1971	RIR	.554	3.395
	SCWL	.173	4.006
	RIR x SCWL	.277	4.785
Apr. 5, 1971	RIR	.573	3.835
	SCWL	.141	3.765
Apr. 19, 1971	RIR	.548	3.495
	SCWL	.158	3.946
Apr. 26, 1971	RIR	.563	3.785
	SCWL	.204	3.550
May 3, 1971	RIR	.554	3.445
	SCWL	.163	3.895
May 10, 1971	RIR	.574	3.650
	SCWL	.171	3.775
	RIR x SCWL	.294	4.455

Appendix Table III. Fertility in SCWL and RIR x SCWL females inseminated with RIR, SCWL, and RIR x SCWL semen

Males Used	Females Inseminated	No. Females	No. Eggs	No. Fertile
RIR	SCWL	15	155	51(32.9)
SCWL	SCWL	15	153	85(55.6)
RIR x SCWL	SCWL	16	157	102(65.0)
MIX[1]	SCWL	17	178	84(47.7)
RIR	RIR x SCWL	20	193	67(34.7)
SCWL	RIR x SCWL	18	168	91(54.2)
RIR x SCWL	RIR x SCWL	17	155	109(70.5)
MIX[1]	RIR x SCWL	17	147	59(40.1)

[1]Equal numbers of RIR and SCWL sperm.

Appendix Table IV. Fertility of turkeys inseminated with turkey semen

Days After A.I.	No. Eggs	No. Fertile (%)
2-8	38	34(89.5)
2-15	73	60(82.2)
2-22	98	78(79.6)
2-29	121	90(74.4)
2-8	38	34(89.5)
9-15	35	26(74.3)
16-22	25	18(72.0)
23-29	23	12(52.2)

Appendix Table V. Recovery of sperm from RIR x SCWL females following inseminations with rooster, turkey and pheasant semen[1]

Semen Source	Section of Tract Flushed	Time Between Insemination and Sperm Recovery: 30 Min. No. Sperm Observed ±S.E.[2]	30 Min. No. Sperm Est'd.	60 Min. No. Sperm Observed ±S.E.	60 Min. No. Sperm Est'd	90 Min. No. Sperm Observed ±S.E.	90 Min. No. Sperm Est'd
Rooster	Vagina	231.3±27.510	77,333.3	183.7±13.932	62,787	195.3±25.563	65,900
	UV	10.0± 3.464	350.0	8.7± 1.667	303.3	19.3± 2.848	676.7
	Uterus	1.3± 0.667	16.7	2.0± 1.000	25.3	3.0± 1.000	37.7
	Magnum	0.3± 0.333	5.0	2.0± 0.577	25.3	0.7± 0.333	8.3
	Infundibulum						
Turkey	Vagina	285.0±33.292	96,900.0	217.3±25.887	73,893.3	223.3± 8.838	75,933.3
	UV	12.3± 2.963	431.7	11.0±2.082	385.0	16.7± 2.333	583.3
	Uterus	4.0±1.155	50.0	4.0±0.000	50.0	3.3± 1.763	41.7
	Magnum	2.7±0.882	164.7	1.7±0.882	21.0	2.0± 1.155	25.0
	Infundibulum	2.0±1.155	25.0	2.3±0.667	27.7	5.3± 2.667	66.7
Pheasant	Vagina	204.3±20.342	69,473.3	208.7±20.003	70,946.7	209.7±12.032	71,286.7
	UV	11.3± 2.603	396.7	13.3± 1.764	466.7	14.3± 1.856	501.7
	Uterus	3.3± 0.667	41.7	1.7± 0.882	21.0	2.3± 0.882	29.7
	Magnum	1.7± 1.201	21.0	1.7± 0.667	21.0	1.7± 0.882	21.0
	Infundibulum	1.3± 0.667	16.7	2.3± 1.201	29.3	4.3± 1.041	54.3

[1]Sperm cell numbers were kept equal per insemination.

[2]Observations based on a mean of 3 birds.