THE EFFECTS OF SPERM DOSE, SEMEN QUALITY, AND RETROGRADE SPERM BLOCKAGE ON ACCESSORY SPERM NUMBER AND EMBRYO QUALITY IN THE ARTIFICIALLY INSEMINATED BOVINE

by

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(ABSTRACT)

This study was designed to: 1. Determine the effects of sperm dose and retrograde sperm blockage on mean number accessory sperm/ova. 2. Evaluate the relationship between mean number accessory sperm/ova and fertilization status/embryo quality. 3. Determine if mean number accessory sperm/ova or embryo quality are affected by semen quality. 4. Compare the percentage of morphologically normal accessory sperm with the percentage of normal cells in the inseminate. Using excised reproductive tracts, a French insemination rod housed in a 24-gauge Foley catheter was determined to be effective in blocking retrograde flow of semen following insemination. In a preliminary study, blocked vs conventional inseminations (control) were made using average quality frozen semen at 20 x 10^6 sperm/dose. Although not different ($P > .1$), the mean number accessory sperm/ovum was 20 ± 40 ($n = 24$) and 13 ± 28 ($n = 26$) for the blocked and control methods, respectively. In Expt. 1, the conventional (control) and blocked system were again compared in a 2x2 factorial using low quality semen at 20 and 40 x 10^6 sperm/dose. Mean number accessory sperm/ova was not affected by dose, blocking, nor the interaction. Embryo quality was negatively affected by blocking ($P < .1$), and unaffected by sperm dose. In Expt. 2, embryo quality and accessory sperm numbers were unaffected by a 40 x 10^6 sperm dose of either average or below average quality semen. However, embryo quality tended to be improved by the average quality semen. Accessory sperm were significantly enriched with morphologically normal cells when compared with those in the inseminate ($P < .01$). Viable quality embryos (poor thru excellent) had the highest mean number accessory sperm/ovum (16.2 ± 28.9), most unfertilized ova (UFO) contained zero

accessory sperm (.27 ± .83) and degenerates embryos were intermediate in number (5.4 ± 8.7). The relationship between embryo quality and accessory sperm number appears to vary in response to semen quality.

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Introduction

Recent studies of fertility in the female bovine have provided information that suggests a major cause of reproductive failure may be related to insufficient numbers of quality sperm present in the oviduct at the most opportune time for fertilization. Although only one spermatozoon can fertilize the ovum, many spermatozoa are often trapped in the outer covering of the egg (the zona pellucida). These spermatozoa are referred to as "accessory sperm". Although accessory sperm were not directly involved in the fertilization process, they do represent a population of spermatozoa that were able to traverse the barriers of the female reproductive tract, gain access to the oviduct and the site of fertilization, and attach to or partially penetrate the zona pellucida of the ovum. Thus, differences in accessory sperm may reflect actual numbers of sperm in the oviduct at the time of fertilization. If so, further study and observation of these populations and factors governing their control may yield a better understanding of optimum conditions for sperm transport and fertilization.

Evidence suggests a positive correlation between numbers of accessory sperm and both fertility (Hunter & Wilmut, 1984; Weitze et al., 1988) and embryo survival (Saacke et al., 1988b).

Following artificial insemination of cattle, approximately 90% of the spermatozoa are lost due to retrograde flow (Mitchell et al., 1985). It is apparent that any method or technique associated with

artificial insemination that could reduce retrograde loss and/or enhance sperm retention and transport in the cow could increase accessory sperm numbers and perhaps fertilization and embryo survival rates.

These experiments were designed to: 1. Determine the effect of blockage of retrograde sperm loss at insemination and sperm dosage on accessory sperm numbers per embryo/ovum. 2. Establish the relationship of accessory sperm numbers with fertilization status of the ovum and embryo quality. 3. Determine if accessory sperm numbers or fertilization status/embryo quality are affected by semen quality. 4. Compare the proportion of morphologically normal cells in the inseminate with that in the zona pellucida of 6 to 7 d embryos/ova (accessory sperm).

Literature Review

Distribution of Spermatozoa in the Female Genital Tract

Retrograde Sperm Loss

Retrograde sperm loss refers to spermatozoa that are transported caudally from the site of semen deposition in the female reproductive tract. This loss occurs in both natural (El-Banna & Hafez, 1970) and artificial matings (Larsson & Larsson, 1985) and, in some cases, can account for approximately ninety percent of the inseminate (Mitchell et al., 1985). Mitchell and coworkers also showed that the majority of this loss can be attributed to spermatozoa that are trapped or caught and swept away in cervical mucus which is present in large volumes in the female bovine genital tract at the time of estrus. Smooth muscle contractions, which are elevated in the female reproductive tract during this stage of the estrous cycle (VanDemark & Hays, 1952), may also play a significant role in stimulating retrograde sperm loss (Suga & Higaki, 1971).

Suga and Higaki (1971) deposited 300 x 10^6 spermatozoa into the uterine body of cows and necropsied the animals between 3 min and 5 h later. Sperm were recovered by scraping the lining of the reproductive tract with the edge of glass slides. Although the numbers of sperm recovered by this procedure probably did not approach the actual numbers present, the data clearly demonstrated changes in sperm distribution after insemination. Within 30 min of insemination, most of the sperm could be recovered from the uterus. At 30-60 min following insemination, most of the sperm population had moved caudally and were recovered from the cervix and vagina.

Mitchell and coworkers (1985), while studying the distribution and retention of spermatozoa with acrosomal and nuclear abnormalities in the female bovine genital tract, observed that only 6.3% of the inseminate could be recovered 12 h following artificial insemination. A second experiment was conducted to determine the extent of retrograde sperm loss from the female reproductive tract following artificial insemination. Three pairs of Holstein cows were artificially inseminated with 420 x 10^6 spermatozoa in a .5 ml dose of fresh semen. The inseminate was deposited in the uterine body. All discharged mucus and urine was collected for 12 h following insemination and examined for presence of spermatozoa. At 12 h after insemination, cows were slaughtered, genital tracts were ligated and removed, and sperm retention and distribution evaluated. An average of 73% of the total number of spermatozoa inseminated were recovered from each cow. Approximately 61% of the inseminate was recovered from discharged mucus or 83% of the total number of sperm recovered. Cumulatively, this loss was nearly complete within 8 h of insemination and greater than half of the total number of spermatozoa were recovered from cervical mucus within 6 h of insemination. Only 6.5% of the inseminate was actually retained in the reproductive tract 12 h following insemination. When distribution of spermatozoa retained in the tract was evaluated, Mitchell et al (1985) observed that greater than 90% were found in the vagina. With respect to site of semen deposition being the uterine body, this marked proportion in the vagina again suggested that large numbers of spermatozoa were eliminated from the gamete transport mechanisms of the female due to retrograde sperm loss. Overall, results from this experiment suggest that as much as 90% of the

artificially inseminated spermatozoa are lost from the female reproductive tract as a result of retrograde flow.

A similar experiment conducted by Nelson and coworkers (1987) produced results almost identical to those of Mitchell. In this experiment, 72.9 x 10^6 cells of frozen-thawed semen were deposited into the uterine body of 11 cows. Cervical mucus and urine were again collected for 12 h following insemination at which time animals were slaughtered. In total, 72% of the inseminate was recovered within 12 h following insemination. Sixty-eight percent of the inseminate was recovered from cervical mucus or retained within the vagina. This number was found to be inversely proportional to the numbers of spermatozoa that actually reached the site of fertilization in the oviduct, again suggesting that large numbers of spermatozoa are lost from the female bovine genital tract following insemination. The authors also suggested that reduction of retrograde sperm loss could increase numbers of sperm gaining access to the oviducts and available for fertilization.

Results of Mitchell's and Nelson's studies are supported by results of experiments performed by several other researchers. Larsson and Larsson (1985) observed that only 14.6% of the total inseminate could be recovered from the reproductive tract of artificially inseminated heifers 2 h following semen deposition. At 12 h, only .6% of the inseminate could be recovered. Dobrowolski and Hafez (1970) slaughtered heifers at 1,8, and 24 h post-insemination and observed sperm recovery rates of 13.4%, 3.8%, and .9%, respectively.

Lightfoot and Restall (1971) recovered only 15% of the inseminate from the reproductive tract of ewes 2 h after insemination. Similar figures regarding retrograde sperm loss were observed in the ewe by Quinlivan and Robinson (1969) and Allison (1972).

First et al., (1968) observed similar losses of spermatozoa from the genital tract of yearling sows following artificial insemination. The number of sperm recovered from the uterus at 2 h was only 22% of the number recovered at 15 min following semen deposition.

Collectively, these studies support the concept that large numbers of spermatozoa are expelled from the vagina of domestic animals due to retrograde flow immediately following insemination.

Conversely, Tilbrook and Pearce (1986) recovered 82% of the inseminate from ewe genital tracts at 3 h following insemination but noted that losses increased rapidly after this time with only 18% and 10% being recovered at 9 and 12 h, respectively. They also observed that the proportion of sperm lost from the vagina due to retrograde flow was not affected by stage of estrus, sperm motility, sperm concentration, or inseminate volume. Morton and Glover (1974), however, found that when the number of spermatozoa in the inseminate was held constant, more sperm could be recovered from the genital tract of rabbits when smaller inseminate volumes were used. They also observed a linear relationship between numbers of sperm recovered from the oviducts and the number of spermatozoa in the inseminate; however, when inseminates contained large numbers of spermatozoa, proportionately fewer sperm were recovered from the entire genital tract.

Hawk and Conley (1971) made an attempt to reduce retrograde sperm loss and increase sperm retention in the female ovine genital tract. Reproductive tracts of ewes in the treatment group were ligated at the vulvovaginal junction at the time of insemination. Ewes were slaughtered 24 h later and the number of spermatozoa in each segment of the tract was estimated. Sixty-two percent of the total inseminate was recovered from the ewes with ligated tracts at 24 h following insemination. Less than 1% of the inseminate was recovered from the control ewes with unligated tracts. Despite the apparent increase in sperm retention, fewer spermatozoa were found in the oviducts of ligated tracts than those of controls. They suggested that this reduction in numbers of sperm at the site of fertilization could possibly be due to a shift in the direction of uterine contractions caused by the ligature itself. They compared these results with those of Hawk (1970) and Conley and Hawk (1970), where insertion of a plastic spiral, or intra-uterine device (I.U.D.), into the ovine uterine body caused uterine contractions to be propagated towards the cervix rather than the oviducts, totally inhibiting sperm transport to the oviducts.

In the rat and porcine, the male produces a gel or plug following natural mating that may partially block retrograde sperm loss and increase sperm retention. Matthews and Adler (1978) observed that the mean number of sperm transported into the uterine body of rats was correlated with the proportion of the vaginal-cervical junction filled with the vaginal plug; i.e., the closer the fit of the vaginal plug with the diameter of the vagina, the greater the sperm transport through the cervix and into the uterine body. These data suggests that female rats with loose fitting vaginal plugs would allow more spermatozoa to be lost due to retrograde flow and thus eliminated from the sperm transport mechanisms.

Gallagher and Senger (1989) studied the rate of retrograde sperm loss in virgin Holstein heifers in relation to site of semen deposition. Eight hours following standing estrus, heifers were inseminated in 1 of 3 locations: 1. Uterine horns. 2. Uterine body. 3. Directly into the cervix, 2 cm anterior to the external cervical os. A 1 ml aspirate of vaginal mucus was collected at 1 h intervals for 8 h following insemination. Aspirates were liquefied and concentrations of spermatozoa evaluated using a hemocytometer. No difference was observed in the rate of retrograde sperm loss when semen was deposited in the uterine horns or in the uterine body. Recovery of spermatozoa from the vagina was significantly higher when semen was deposited in the cervix as opposed to the sites of deposition in the uterus. The results suggest that retrograde movement of spermatozoa was similar when semen was deposited past the cervix and into the uterus but twofold greater following cervical deposition.

Other Factors Responsible for Sperm Loss

Within 12 h following insemination, Mitchell et al. (1985) and Nelson et al. (1987) recovered approximately 72 to 73% of the spermatozoa inseminated; thus, approximately 27% of the inseminate was unaccounted for. There are two possible explanations for this loss of sperm from the female genital tract. Many spermatozoa may disappear from the female genital due to phagocytosis by

leucocytes following insemination. Mitchell observed spermatozoa being engulfed by leucocytes but did not attempt to quantitate this phenomenon.

Howe and Black (1963) observed that the number of leucocytes in the female reproductive tract of calves increased significantly between 4 and 8 h following vaginal insemination of bacteria-free semen. Mattner (1968) observed a similar response in increased numbers of leucocytes after insemination of goats and cows. Although no experiments have been performed to quantitate the actual percentage of the inseminate that may disappear due to phagocytosis, Mattner (1969) suggested that the likelihood that an individual sperm cell would become engulfed and digested by leucocytes is correlated with sperm motility i.e. - motile sperm are more likely to come in contact with leucocytes than immotile spermatozoa.

A second possible explanation for the disappearance of sperm from the female genital tract following insemination is peritoneal migration. Larsson and Larsson (1985) found spermatozoa in the infundibulum of heifer oviducts both 2 and 12 h following insemination. Spermatozoa have been observed at the ovarian end of the oviduct within minutes of semen deposition in rabbits (Overstreet and Cooper, 1978a), human (Settlage, Motoshima and Tredway, 1973), sheep (Mattner, 1963) and cattle (VanDemark and Moeller, 1951). Mattner and Braden (1963) observed sperm distribution in the reproductive tracts of six ewes that had one open and one occluded oviduct. The occlusions were the result of ovariectomies performed 2 yr earlier. In all six ewes, the number of spermatozoa in the oviducts 4 h following insemination was found to be higher in the occluded tubule than in the open tubule. Larsson (1986) observed spermatozoa in distal portions of heifer oviducts even after segments proximal to the uterotubal junction were ligated and resected prior to insemination. These experiments suggest that some spermatozoa may pass through the oviducts and are lost to the abdominal cavity following insemination.

Sperm Transport Mechanisms

Efficient sperm transport in cattle is controlled by complex endocrine interactions in the female genital tract during estrus. Estrogen, from the maturing follicle, promotes the secretion of sialomucin (Mattner, 1973; Heydon & Adams, 1979) from the epithelial cells of the cervix. The secretory pattern of this highly fluid mucin in relation to the cervical mucosa appears to facilitate sperm transport through the narrow basal canals of the cervix (Mullins & Saacke, 1989).

Estrogen also stimulates muscular contractions of the reproductive tract (Hawk, 1975; Ruckebusch & Bayard, 1975) which can result in the transport of killed spermatozoa to the oviducts of cows (VanDemark and Moeller, 1951), sheep (Mattner, 1963), and pigs (First et al., 1968) within minutes of insemination. The number and strength of contractions in the genital tract of cows (Ruckebusch & Bayard, 1975) and ewes (Hawk, 1975) increases at the time of estrus. VanDemark and Hays (1952) also observed that the number and strength of uterine contractions in the cow increased for several minutes following mating.

Uterine contractions appear to reverse directions as ovulation approaches. Döcke (1961) inserted two balloons into the cow uterus to measure strength and direction of motility patterns. He concluded that rhythmic activity was maximal during estrus and that the contractions during the early portion of this period initiated at the cervix and were propagated towards the oviducts. These peristaltic contractions then reversed directions near the end of estrus.

Oxytocin, which is most often associated with milk let-down in the cow, has also been observed to stimulate uterine contractions. Hays and VanDemark (1951) found that natural mating and certain techniques of artificial insemination could stimulate milk let-down. A study of the reciprocal interaction demonstrated that manipulation of the mammary gland, even in nonlactating cows, resulted in increased uterine activity (VanDemark and Hays, 1951). Hays and VanDemark

(1953) demonstrated that oxytocin had direct effects on uterine contractions and that these effects could be negated by injections of epinephrine.

Other hormones and compounds tested regarding their effect on uterine motility and sperm transport include: progestagen (Hawk & Conley, 1971); ergonovine (Hawk & Cooper, 1984; Hawk & Conley, 1985), prostaglandin $F_{2\alpha}$ and phenylephrine (Hawk & Conley, 1985). Results suggest that the ability of a compound to increase the number or strength of uterine contractions was not always correlated with an increase in transport or retention of spermatozoa, suggesting that factors other than uterine motility are also involved in efficient sperm transport systems.

The origin and direction of uterine contractions appears to be an important factor involved in the sperm transport system. In a review article, Hawk (1987) suggested that increased muscular activity in the uterine horns early in estrus could be associated with transport of sperm through the uterus during the rapid and sustained phase of sperm transport. He also suggested that increased activity near the uterotubal junction later in estrus may be responsible for transport of sperm into the caudal isthmus of the oviduct and may also be related to retention of spermatozoa in this segment.

Quantification of Sperm Numbers in the Female Genital Tract

Mattner (1968) was apparently the first to attempt to quantitate sperm distribution in the female reproductive tract of cattle following insemination. Five cows were mated naturally and slaughtered 19 to 22 h later. Reproductive tracts were ligated at several locations immediately following necropsy. Spermatozoa were recovered by flushing the various segments of the ligated tracts. A distinct gradient in sperm numbers was observed, decreasing from several million in the cervix to several thousand in the oviducts. Mattner also performed this experiment on goats and observed similar results.

El-Banna and Hafez (1970) conducted a similar experiment evaluating sperm distribution in rabbits and cattle following artificial insemination. Twelve Hereford heifers were inseminated at the external cervical os with a known volume and concentration of semen. Heifers were slaughter at either 16 or 40 h following insemination at which time genital tracts were ligated and flushed to recover spermatozoa. Rabbit does were handled in a similar manner but were slaughtered at 1, 4, 8, 12, 16, 20, 24, 36, 48, and 72 h postinsemination. The maximum number of spermatozoa recovered from the oviducts of heifers was 17,025 at 16 h compared to 4,230 at 40 h following insemination. The number of spermatozoa recovered from rabbit oviducts maximized at 8 to 12 h post-insemination despite the fact that the total number recovered from the entire tract was consistantly decreasing with time. This study was consistent with the findings of Mattner (1968) in that the number of sperm reaching the site of fertilization in the oviducts was in the thousands while the number inseminated was in the millions to billions.

Dobrowolski and Hafez (1970) also found maximum numbers of spermatozoa were recovered from the oviducts at 8 h following insemination of Hereford heifers. However, the maximum number recovered in the study (2×10^6) was much greater than that of previous studies.

Movement of Spermatozoa into the Oviducts

Fertilization failure in cattle is usually a result of failure of spermatozoa to contact the oocyte rather than unfertilizability of ova (Hawk, 1987). Although a large percentage of spermatozoa are lost from the female reproductive tract following insemination due to retrograde flow and phagocytosis, the distribution of the population of sperm which are retained is of vital importance to a more complete understanding of the sperm transport mechanism and reasons for fertilization failures.

As mentioned earlier, various researchers working with several different species have observed spermatozoa in the oviducts within minutes of fertilization. However, evidence which suggests that

these early arriving spermatozoa are directly involved in the fertilization process has yet to be presented. Quite to the contrary, Overstreet and Cooper (1978a) observed that most of the spermatozoa gaining access to rabbit oviducts within minutes of fertilization were immotile and greater than 90% had damaged membranes overlying the acrosome. These spermatozoa were located almost exclusively in the upper ampullary region of the oviduct and were eventually voided into the peritoneal cavity. Thus, they had no direct role in the fertilization process. The authors described this phenomenon as the rapid transit phase of the sperm transport system and suggested this to be a result of muscular contractions of the female reproductive tract.

The population of cells from which the fertilizing spermatozoa are derived, arrive at the lower isthmus of the oviduct during the sustained phase of sperm transport (Overstreet & Cooper, 1978b). There is general agreement in the literature that this phase of sperm transport requires a minimum of 6 h and extends to approximately 12 h following insemination with some species variation (Hawk, 1987). Although, the actual numbers of spermatozoa estimated to reach the oviducts during this period have differed significantly, many researchers have consistently recovered approximately 20,000 spermatozoa per oviduct (Overstreet and Cooper, 1978b).

Spermatozoa reaching the lower isthmus during the sustained phase of sperm transport are apparently restricted from further migration through the oviduct (Harper, 1973). Overstreet and Cooper (1975) found that spermatozoa in this region exhibit a depressed motility pattern. Overstreet and Cooper (1978b) suggested that this region of the oviduct plays a principal role in regulating the final stages of sperm transport in the rabbit. Suarez (1987) observed what appeared to be two mechanisms of retaining sperm in the oviduct of mice: immobilization and epithelial attachment. Columns of immotile sperm were seen in the lower isthmus and motile sperm in this region appeared to adhere to epithelial cells. Overstreet and Cooper (1979) observed that, in the absence of ovulation, the numbers of sperm reaching the upper regions of the oviduct was significantly reduced despite the fact that numbers in the lower isthmus were the same as for an ovulatory, mated female. This observation suggested that function of the lower isthmus may be regulated by ovulation.

Wilmut and Hunter (1984) mated heifers early in estrus and ligated the reproductive tracts at the uterotubal junction at 6, 8, 10 or 12 h after mating. When ligations were performed at 6 h, only 1 of 11 ova became fertilized. Fertilization rates increased as the time between insemination and tubal-ligature increased with a maximum fertilization rate of 85% occurring in the group that was ligated at 12 h. Accessory sperm numbers per ovum also increased as time from insemination to ligature increased. They concluded that the population of spermatozoa capable of fertilization is established in the oviducts during a period that is not less than 6 h.

In a subsequent study, Hunter and Wilmut (1984) also described the build up of a reservoir of sperm in the lower isthmus of cattle. Cows were inseminated within 8 h of the onset of estrus. Approximately 2 cm of oviduct was ligated and transected proximal to the uterotubal junction at 16 to 36 h post-mating. Cows that had not ovulated at the time of transectiong resulted in only 3 of 14 ova being fertilized. In animals that did ovulate prior to transection, 7 of 8 ova were fertilized. The authors described the lower isthmus as a "functional sperm reservoir" that is drawn upon at the time of ovulation resulting in a subsequent redistribution of the spermatozoal population within the oviduct.

A similar phenomenon, involving the arrest of spermatozoal activity and reservoir property of the caudal region of the oviduct has been observed in gilts (Hunter, 1984) and ewes (Hunter & Nichol, 1983).

Accessory Sperm

Accessory Spermatozoa and the Zona Reaction

Accessory sperm are trapped in the outer vestments of the ovum at the time of fertilization due to a physiological change in the structural properties of the zona pellucida. This change is known as the "zona reaction" and is initiated when the fertilizing spermatozoon comes in contact with the plasma membrane (vitelline membrane) of the egg. This reaction blocks further penetration of spermatozoa already in the zona and prevents later arriving sperm from binding to or penetrating the zona pellucida. The first report of such a reaction was described by Fol (1877) in sea urchins and starfish.

Unfertilized ova contain small vesicles uniformly distributed directly beneath the vitelline membrane known as corticle granules (Moser, 1939). Moser observed that these granules disappeared from the cortical region of the vitellus in Arbacia eggs just prior to the elevation of a "fertilization membrane". Austin and Braden (1956) were the first to suggest that the contents of these granules, when released into the perivitelline space after fertilization (Austin, 1956; Szollosi, 1967), could modify the zona pellucida and render it less penetrable to spermatozoa. Barros and Yanagimachi (1971) and Gwatkin et al., (1973, 1976) presented experimental evidence, in hamster and mouse oocytes respectively, to support the theory that once the fertilizing spermatozoon comes in contact with the vitelline membrane of the ovum, corticle granules are released, initiating the zona reaction. The authors also observed that this reaction was completed in 8 to 15 min and could be blocked or reversed by trypsin inhibitors. Barros and Yanagimachi (1972) demonstrated that the second block to sperm penetration, which occurs at the vitelline membrane, requires 2 to 3 h for completion in zona-free hamster eggs. Wolf and Hamada (1977) demonstrated, in vitro, that both the zona reaction and the vitelline block can be initiated in mouse eggs with cortical granule exudate.

The zona reaction and the block at the vitellus are important steps in regulating the fertilization process. Without them, many ova would allow more than one sperm to penetrate and thus result in polyspermic eggs. This condition is detrimental to embryonic development and usually results in abnormal embryogenesis or early embryonic mortality. Wolf (1981) and Sathananthan & Trounson (1982) observed that the zona plays a major role in the mechanism that prevents or blocks polyspermy.

Conversely, increased zona resistance to sperm penetration can lead to decreased oocyte fertilizability. De Felici & Siracusa, (1982) using in vitro matured cumulus-free mouse oocytes, observed that "spontaneous" hardening of the zona pellucida can occur. The authors noted that this process was delayed when an intact cumulus complex was present with the oocyte. They suggested that this spontaneous hardening occurs as a result of the absence of a compound secreted by the cumulus cells and not as a result of the cortical reaction; however, they did not exclude the possibility that some premature cortical granule release could have occurred in their culture system. Aged mouse oocytes, in vivo or in vitro, (Wolf & Hamada, 1976; Longo, 1981; Gianfortoni & Gulyas, 1985) have been observed to display an increased resistance to sperm penetration and are less susceptible to proteolytic digestion.

Trounson & Webb (1984) and Mahadevan et al., (1983) suggested that impairment of sperm-zona interaction could be a contributing factor in idiopathic human infertility. This idea is supported by observations which suggest that the zona pellucida of human oocytes undergoes important maturational changes which render it more susceptible to sperm penetration (Trounson et al., 1982; Zenzes et al., 1985; Tesarik et al., 1988).

Using in vitro matured and fertilized human oocytes, Tesarik et al., (1988) observed that a higher percentage of accessory sperm penetrated the zona pellucida of Metaphase II ova (27%) compared to Metaphase I ova (8%). In this experiment, no oocytes that were in Metaphase I at the time of insemination became fertilized, while 98% of ova inseminated at Metaphase II were fertilized. Electron microscopy of Metaphase I ova detected a compact and homogeneous appearance of the

zona pellucida distinctly demarcated against the cumulus oophorus. Metaphase II oocytes were described as being porous in appearance with wide slits and a poorly delineated outer boundary. Secretions from the surrounding cumulus cells appeared to invade the slits and porous structure of the zona. The author suggested that these secretions could be "softening factors" that affect the penetrability of the zona. Secretory activity was also noted in the corticle region of Metaphase I and II oocytes. Granules approximately the same size but slightly lower in electron density were observed to be released into the perivitelline space in a slow continuous fashion. It was suggested that these granules, cortical granules or not, could possibly be 'hardening factors' that would reduce zona penetrability. Tesarik suggested that secretions from the cumulus cells of mature oocytes ('softening' factors) antagonized the effect of the 'hardening' factors secreted by the oocyte during the early stages of maturation.

While studying fertility problems of a particular strain of inbred mice (KE), Wabik-Sliz (1979) observed corticle granules of this strain being released prematurely following ovulation. Although sperm were able to penetrate the zona of these oocytes, most were often trapped in the perivitelline space and did not fertilize the egg. She suggested this premature release of granules initiated a block to sperm penetration at the level of the vitelline membrane.

Chang and Hunt (1956) observed that the zona pellucida of unfertilized rat and rabbit eggs were more readily digested by proteolytic enzymes than fertilized ova of these species. This suggested a biochemical change in the constituents of this structure.

Accessory Sperm and Their Relation to Fertility

The amount of literature available regarding studies of accessory sperm and their relation to fertility is limited. However, several studies suggest that the number of accessory sperm per ovum appears

to be positively correlated with conception rates (Hunter and Wilmut, 1984; Weitze et al. 1988) and embryonic survival (Saacke et al., 1988b).

Using frozen-thawed boar semen, Weitze and coworkers (1988), established a highly-significant response of accessory sperm per embryo to addition of seminal plasma to diluted semen. Thirty-six gilts were divided into two groups, 24 were inseminated with 2 x 10^9 and 12 with .5 x 10^9 spermatozoa. Each of these groups were then divided equally into subgroups which received a pretreatments of either 60 ml seminal plasma or 60 ml semen extender. The number of accessory sperm per ovum was significantly higher in the two groups that received the seminal plasma pretreatment. The percentage of fertilized embryos recovered was also higher in the plasma treated groups. Although not statistically significant, accessory sperm numbers per embryo were higher in embryos recovered from gilts inseminated with the higher sperm dose. Mean number of accessory sperm per ovum for the higher dose was 85.2 and 25.9 with and without seminal plasma pretreatment respectively, while the lower dose averaged 69.6 and 8.6, respectively.

Saacke and coworkers (1988b) found that both unfertilized eggs and early embryonic deaths were related to too few sperm penetrating the egg (accessory sperm). They postulated that when decreased numbers of spermatozoa were present in the oviduct and/or sperm transport mechanisms of the female were inefficient (such as in the superovulated cow), it appeared that abnormal spermatozoa were more likely to penetrate and fertilize due to less competition with normal sperm. Thus, lower competition increased the frequency of fertilization by abnormal sperm and caused lower conception rates and increased embryonic mortality.

Hawk and coworkers (1988), found fertilization rates to be near 100% in superovulated cattle when high doses of fresh semen (4.4 billion) were inseminated and only 53% when lower doses of frozen semen (70 million) were used. This suggests that the low fertilization rates typically observed in superovulated cows is not due to an immature or defective oocyte being released from the hormonally stimulated follicles; but, more likely a result of an impairment of the sperm transport mechanisms of the female due to this hormonal manipulation. The proportion of embryos con-

taining accessory spermatozoa was also found to be greater in cows inseminated with high doses of fresh semen (32%) than with the lower dose of frozen semen (12%); however, this difference did not prove to be significant.

While evaluating the effects of unilateral, cornual insemination on fertilization rates of single ovulated cows, Hawk and Tanabe (1986) discovered first-service cows had significantly higher numbers of accessory sperm per ova (mean = 40) when compared to repeat breeders (mean = 19). Also, accessory sperm were present in 98% of ova recovered from first-service cows but in only 77% of ova from repeat-breeders. In superovulated cows, the proportion of cleaved ova containing accessory sperm was also higher for first-service cows (18%) than for repeat-breeders (11%; P = .15). This study suggested a strong correlation between fertilization rate and accessory sperm numbers.

Segerson and Libby (1981) studied the effects of selenium and vitamin E treatment on fertilization rate and accessory sperm number in Charolais cows. Selenium deficiencies are often associated with decreased fertility in bovine populations. All cows were considered low in selenium status prior to the beginning of the experiment. Control and treatment cows were fed the same selenium deficient diet during the experimental period. Cows in the treatment group received intramuscular injections of selenium and vitamin E at 14 d intervals throughout the study. No difference was observed in the percentage fertilized ova recovered from the two groups; however, cows that received selenium and vitamin E treatment tended to have higher numbers of accessory sperm per embryo/ovum (35.6 ± 7.2) than did the control cows (24.8 ± 7.7). Again, as observed by other researchers, these values were not statistically significant due to the high variation in number of accessory sperm per embryo/ovum.

Saacke and coworkers (1986) presented evidence which suggested that accessory sperm per embryo/ovum was correlated with fertilization status/embryo quality. They found that unfertilized ova had basically zero accessory sperm, degenerates and poor quality morulas were intermediate in number while transferable quality morulas and blastocysts had the highest mean number accessory

sperm per embryo/ova. If in fact this is the case, embryo survival should response to changes in accessory sperm number.

The evidence presented thus far suggests that accessory sperm numbers could possibly be used as an indirect measure of fertility; however, due to the high variation in absolute numbers of accessory sperm found in previous experiments, application of this theory at the present time is limited. While studying sperm transport and retention in the female genital tract, Mitchell and coworkers (1985) found high variation in the numbers of sperm that reach the lower isthmus of the oviduct. This could possibly explain the high variation in accessory sperm numbers found by other researchers. Mitchell also found that retrograde loss of artificially inseminated spermatozoa was near 90%. This would suggest that any method or technique of A.I. that could improve sperm retention and reduce retrograde loss might stand a good chance of improving reproductive performance by increasing accessory sperm numbers.

Semen Quality and It's Relation to Fertility

Semen quality may be divided into three major components; viability, morphology and functionality. Viability reflects the life processes of spermatozoa and is most often measured as percent motile cells. Morphology refers to the shape of the sperm and reflects variations in the spermatogenic processes. Functionality refers to the ability of spermatozoa to undergo capacitation, attach to and penetrate the zona pellucida of the ova as well as form pronuclei. Most measures of semen quality are correlated to some degree with fertility or conception rates (Linford et al., 1976; Bishop et al., 1954; Saacke & White, 1972).

Sullivan and Elliott (1968) observed that bulls of different fertility levels respond differently to changes in sperm concentration. In their study, low fertility bulls showed the greatest response to

increases in sperm concentration while high fertility bulls displayed the lowest response; suggesting that semen of marginal quality may achieve maximum fertility simply by providing additional numbers of spermatozoa to the inseminate. This may be referred to as a compensable component of semen quality and is usually associated with sperm viability traits. Uncompensable factors affecting semen quality are those that cannot be negated by increasing numbers of spermatozoa in the inseminate and are not recognizable by current methods of semen evaluation (Saacke, 1988b).

A possible example of an uncompensable factor affecting semen quality may be the quality of DNA measurable by the instability of nuclear DNA to heat or acid denaturation as described by Evenson and coworkers (1980). The ability of sperm chromatin to with-stand denaturation has been correlated with fertilization rates (Ballachey et al., 1987). Evenson (1980) also observed that increased heterogeneity of chromatin structure is associated with high proportions of morphologically abnormal spermatozoa. Although direct evidence has yet to be presented, it is possible that spermatozoa with abnormal chromatin structure, but normal morphology and motility, are capable of fertilization but unable to sustain embryonic development; thus, resulting in decreased reproductive performance.

Motility is critical for establishing populations of sperm in the lower isthmus during the sustained phase of sperm transport (Mattner, 1963). Spermatozoa with morphologically abnormal heads have been shown to have progression impaired at the cervix (Mortimer, 1977; Karabinus & Saacke, 1987), uterus (Mitchell et al., 1985), and the uterotubal junction (Krzanowska, 1974). Krzanowska (1974) observed that rat spermatozoa with severe morphological abnormalities were excluded at uterotubal junction but those with subtle abnormalities passed the barriers readily and were capable of fertilizing eggs in vitro (Krzanowska & Lorenc, 1983). Dresdner and Katz (1981) found that spermatozoa with abnormal head shapes displayed an altered motility pattern which may be responsible for a decreased efficiency of sperm transport.

Whether due to an indirect effect of morphology on motility patterns or due to a direct selection process of the female genital tract, it appears that morphologically abnormal spermatozoa are ex-

cluded from access to the oviducts. However, this selection process is incomplete. Saacke and coworkers (1988a) observed accessory spermatozoa in bovine ova with slight or subtle abnormalities. Although accessory sperm were enriched with morphologically normal cells as compared to that of the inseminate, spermatozoa with slight abnormalities were not excluded by the sperm transport mechanisms of the female. Saacke et al. (1988a) suggested that such sperm could possibly fertilize ova but be incapable of sustaining embryonic development. This would represent an uncompensable component of semen quality that would not respond to an increase in sperm dosage.

In vitro fertilization studies using bovine spermatozoa have presented evidence which suggests that sperm from different bulls vary in their ability to penetrate oocytes and to sustain embryonic development (Hillery et al., 1990; Eyestone & First, 1989; Shi' et al., 1990). In vitro performance was also shown to be related to in vivo fertility (Hillery et al, 1990). Miller and coworkers (1982), working with superovulated cows, observed that semen with a high percentage of cratered sperm yielded a lower percentage of fertilized embryos and higher proportions of non-transferable embryos than semen from control bulls. This high percentage of lower quality embryos suggested a male related factor may be partially responsible for early embryonic mortality.

Bearden et al. (1956) and Kidder et al. (1954) observed differences in nonreturn rates due to fertilization failures and early embryonic death when comparing high and low fertility bulls. When low fertility bulls were used to inseminate, fertilization failure was the major factor responsible for returns to estrus. When high fertility bulls were used for mating, fertilization rates approached 100% and the major factor responsible for returns to estrus was early embryonic death. Thus, it appears that variations in semen quality may be responsible for variations in incidence of fertilization failure and male-related embryonic death.

Experimental Rationale

With ninety percent of artificially inseminated spermatozoa being lost from the reproductive tract due to retrograde flow, it is apparent that any method or technique that could reduce retrograde loss of sperm, could increase sperm retention, transport and possibly accessory sperm numbers. In this series of experiments, we will attempt to increase sperm retention and/or numbers of sperm reaching the site of fertilization by blocking retrograde flow at the internal cervical os or by altering sperm dosage. Efficiency of these procedures will be determined by evaluating the mean number of accessory sperm per embryo/ovum.

Accessory sperm numbers may be correlated with fertilization rates and embryo quality. If this is in fact the case, can we alter embryo quality distribution by altering accessory sperm numbers per ovum in response to either blockage of retrograde flow or by altering sperm dose? Whether or not accessory sperm numbers per ova are altered in response to treatments, we will attempt to more clearly establish the relationship between accessory sperm numbers and fertilization status/embryo quality.

Literature from recent studies has presented evidence which suggests that embryo quality and fertility rates are related to semen quality. We will further study this phenomenon by altering semen quality and evaluating responses in embryo quality and accessory sperm numbers. The accessability to the ovum of sperm having abnormal morphology will also be evaluated since abnormals (particularly those involving the head) are negatively related to fertility.

Materials and Methods

Animals

Animals from a herd of sixty mature, nonlactating Holstein and Jersey cows were used repeatedly for each of three experiments. Cows were observed for signs of estrous behavior twice daily (early morning & late afternoon). Cows were considered to be in estrus if they stood to allow mounting by herdmates and only cows exhibiting natural or spontaneous estrus were included in this study. Upon observation of standing, cows were randomly assigned to treatment groups and artificially inseminated approximately 12 h later. The inseminate was deposited directly into the uterine body regardless of treatment or experiment. Embryos were recovered 6 or 7 d following insemination at the late morula to early blastocyst stage of development using nonsurgical flushing techniques.

Embryo Recovery

Prior to flushing, hair was clipped from the tail head near the junction of the sacral and lumbar vertebrae. This area was scrubbed clean with Betadine (Purdue Fredrick Co., Norwalk, CT.) and a spinal epidural of approximately 3.5 ml Lidocaine (Butler Co., Columbus, OH.) was administered in order to reduce rectal contractions. Ovaries were palpated for the presence of a functional corpus luteum to determine the side of ovulation. A 20-gauge Foley catheter was then inserted through the cervix and the cuff inflated at the junction of the uterine body and the internal cervical os, locking the catheter in place. The flushing medium was 500 ml Dulbecco's phosphate buffered saline with 5 ml newborn calf serum and 1 ml of an antibiotic-antimycotic (Gibco Laboratories, Grand Island, N.Y., See Appendix A). Via rectal palpation, fluid was directed to either horn of the uterus by occluding the opposite horn thus blocking the flow of flushing medium into that horn. Approximately 350 ml of medium was directed into the horn ipsilateral to the corpus luteum and the other 150 ml of medium was directed to the opposite horn. Fluid was deposited and recovered from the uterus in increments of 50-100 ml. The recovered flushing medium was transported approximately .5 mile to the laboratory and filtered using an Em-Con embryo concentrator (Immuno Systems, Inc., Spring Valley, WI.). Material trapped by the filter and approximately 30 ml of fluid were transferred to a Petri dish and examined under a dissecting microscope for the presence of an embryo or ovum.

Fertilization Status and Embryo Quality Evaluation

Upon recovery, fertilization status of the ovum and/or embryo quality was immediately assessed. Unfertilized ova (UFO) were designated when the ovum displayed no sign of cleavage and when one or two cell divisions had occurred without apparent nuclear formation in blastomeres when

later examined at 100x under phase contrast microscopy. Embryos which had undergone cleavage and blastomere nuclear development with an apparent arrest of cell division were considered degenerate. These embryos were characterized as having approximately 8 to 16 blastomeres which were clearly dissociating from each other with no compact inner cell mass. Quality of viable embryos was determined according to procedures described by Lindner and Wright (1983). This evaluation was based on compactness and homogeneity of the cell mass and resulted in the designation of the following categories: Excellent, Good, Fair, and Poor. See Appendix B for a more complete description of embryo quality evaluation procedures.

Accessory Sperm Evaluation

Immediately following quality evaluation, the zona pellucida of the embryo/ovum was partially digested at 37°C in a hanging drop slide containing a 3 ul drop of .5 % Pronase (Behring Diagnostics, La Jolla, CA). Prior to preparation, the coverslip received a light smear of petroleum jelly around the edges to prevent evaporation during zona digestion. The embryo/ovum was examined periodically in the hanging drop slide at 400x magnification under phase-contrast microscopy to monitor the zona digestion process. This process required approximately 7 $\pm$ 3 min and was considered complete when the outer edge of the zona pellucida became poorly delineated or irregular in appearance. At this time, the coverslip, with the embryo/ovum, was removed from the hanging drop slide and lowered onto a siliconized slide containing a 5 ul drop of phosphate-buffered saline. Once the two drops of solution were brought into contact with each other, the coverslip was released, gently flattening the zona pellucida and extruding the blastomeres or vitellus. The area containing the flattened zona was then circled with a waterproof marker to aid in relocation after transfer of the slide to the phase contrast and/or differential interference contrast microscopes for quantification of accessory sperm numbers at 400x magnification. Accessory sperm morphology evaluations were conducted under oil immersion at 1000x magnification and only primary abnor-

malities (abnormalities of the sperm head) were considered. Flagellar abnormalities could not be considered since the flagellum was usually absent by this time (day 6).

Retrograde Sperm Blockage

Using excised reproductive tracts, a standard .5 ml French insemination rod encased in a modified 24-gauge Foley catheter was inserted through the cervix and locked into position by inflating the cuff just anterior to the internal cervical os. The catheter was modified by removing the distal end approximately 5 mm from the inflation cuff. The end was then trimmed to apply a slight bevel which would aid in traversing the cervix during insemination procedures. The breeding apparatus was assembled by sliding the modified catheter over the shaft of the loaded insemination rod until the tip of the disposable sheath extended approximately 3 to 4 mm beyond the beveled end of the Foley (Figure 1).

After close examination of the stability of this device and it's ability to block the cervical lumen, this breeding apparatus was presumed to be potentially effective in blocking retrograde flow of spermatozoa following artificial insemination.

In the Preliminary Experiment and Experiment 1, in vivo inseminations were conducted using this breeding apparatus. The block was performed by inflating the catheter cuff with approximately 8 cc of air prior to semen deposition (as described with the excised tracts). The block was locked securely in place by clamping the inflation nipple with hemostats. At the time of semen deposition, the horns of the uterus were grasped (via rectal palpation) while simultaneously pulling the breeding apparatus in a caudal direction to ensure that the tip of the gun was in the proper location to deposit semen into the uterine body and that the inflated cuff was seated at the internal cervical os. Immediately following semen deposition, the shaft of the blocking device was cleaned and dried in

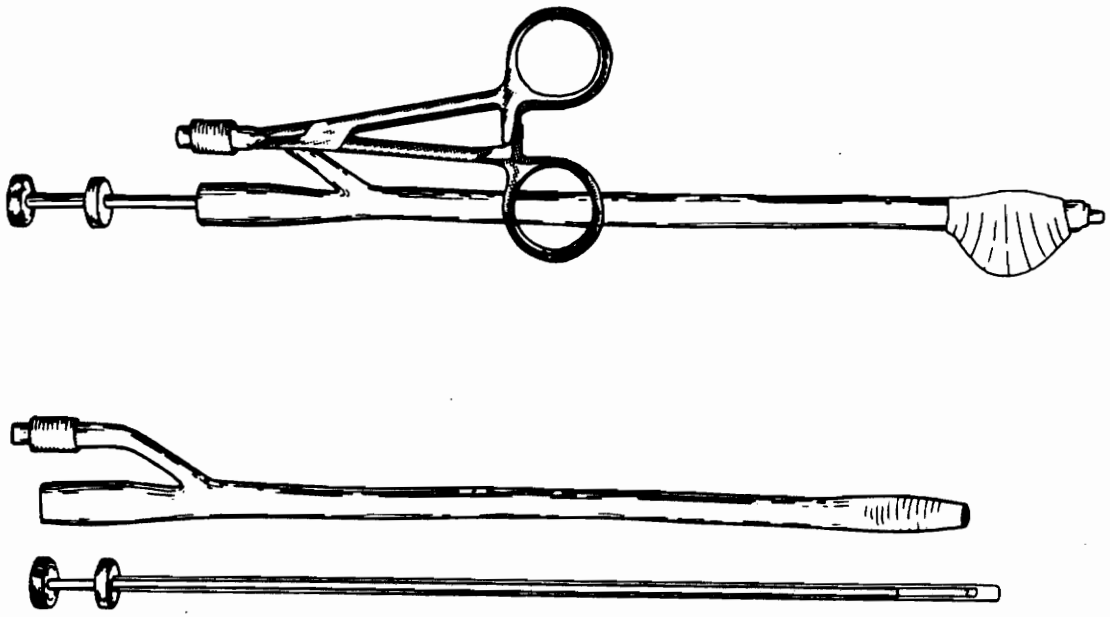

Figure 1. Schematic Drawing of Breeding Apparatus Designed to Block Retrograde Flow at the Time of Insemination

the area adjacent to the vulva and a 30 cm section of a plastic insemination rod was securely taped on a perpendicular axis in a manner such that when the breeding apparatus was released, the cuff would remain snug against the internal cervical os. The tail of the animal was then tied to the neck chain in order to restrict movement that could result in disruption or removal of the blocking device. Cows were restrained in standing position for the duration of the blocking procedure.

Conventional Artificial Insemination Procedures

All inseminations, regardless of experiment or treatment, were of cryopreserved semen frozen in .5 ml French straws. Straws were thawed by plunging in a 35°C water bath for 45 s. According to respective experimental protocals, straws were then diluted and/or loaded into .5 ml French insemination rods. Via rectal palpation, inseminating rods were inserted thru the cervix and semen was deposited in the uterine body approximately 2 to 3 cm anterior to the internal cervical os. Inseminations using the blocking apparatus also followed these procedures; however, the loaded French inseminating rod was encased in the modified Foley catheter prior to insemination.

Preliminary Experiment

A preliminary experiment was designed to test the blocking apparatus and determine its effect on accessory sperm numbers per embryo/ovum. Cows inseminated with the blocking apparatus were restrained in standing position for 1 h following insemination with block in place. Control cows were bred by conventional methods and restrained for 1 h in a similar fashion.

Semen used in this experiment was collected from a single bull and frozen in egg-yolk citrate glycerol extender according to procedures described in a later section (See section on Semen Preparation). Sperm concentration, as determined by hemocytometer count, was 20 x 10^6 cells per .5 ml dose. This semen possessed approximately 40% post-thaw progressive motility and approximately 90% morphologically normal cells and was considered to be of average quality. See Table 1 for a more detailed description of semen quality traits. The inseminate was deposited directly into the uterine body.

Embryo/ovum recovery and accessory sperm evaluations were performed according to procedures previously described.

Experiment 1

Based upon the results of the Preliminary Experiment, Experiment 1 was designed to test the effects of the blocking apparatus on accessory sperm numbers, in a two by two factorial design utilizing one of two dosages (20 x 10^6 or 40 x 10^6 cells per dose) of below average quality semen. Because the standard deviation in accessory sperm numbers per ovum was proportional to the mean in our Preliminary Experiment, a low quality semen was used in this experiment in an attempt to reduce the mean and associated variance in accessory sperm numbers per ovum. Specifically, this semen possessed approximately 20% post-thaw progressive motility and 74% primary abnormalities (See Table 2).

Cows in the treatment groups were bred with the blocking apparatus and restrained in standing position for 3 h with the block in place. Control cows were bred conventionally and restrained in similar fashion. Semen was deposited directly into the uterine body of cows for both control and blocked inseminations.

Table 1. POST-THAW VIABILITY AND MORPHOLOGY CHARACTERISTICS OF CRYOPRESERVED SEMEN USED IN THE PRELIMINARY EXPERIMENT

Viability

	Hours incubation at 37°C		
	0 h	3 h	6 h
Progressive Motility (Percent)	40	20	0
Intact Acrosomes (Percent)	82.0	70.5	55.5

Morphology

	Abnormal Head Shapes					
Normal	Taper	Crater	Asymmetric	Slightly Asymmetric	Long	Pyriform
90	4.5	1.5	.5	3.0	.5	0

(Values represent means of 3 replicates of 100 cells each)

Table 2. POST-THAW VIABILITY AND MORPHOLOGY CHARACTERISTICS OF CRYOPRESERVED SEMEN USED IN EXPERIMENT 1

Viability

	Hours incubation at 37°C		
	0 h	3 h	6 h
Progressive Motility (Percent)	20	20	10
Intact Acrosomes (Percent)	71.0	66.0	62.5

Morphology

	Abnormal Head Shapes					
Normal	Taper	Crater	Asymmetric	Slightly Asymmetric	Long	Pyriform
26	16	8	9	25	14	2

(Values represent means of 3 replicates of 100 cells each)

Semen for the low dose (20 x 10^6) was prepared by diluting the contents of a straw containing 40 x 10^6 cells with an equal volume of clarified egg yolk citrate extender. This extender was frozen the same day from the same batch used to extend and freeze the semen. Dilution was carried out in a 1.5 ml Eppendorf tube at 35°C. Once diluted, the semen was then reloaded by syringe generated vacuum pressure into .5 ml French straws. To duplicate this procedure, semen for the high semen dose (40 x 10^6) was also extruded into 1.5 ml Eppendorf tubes and then reloaded into .5 ml French straws without dilution.

Embryo/ovum recovery and accessory sperm evaluations were performed according to procedures previously described.

Experiment 2

Based upon the results of the Preliminary Experiment and Experiment 1, Experiment 2 was designed to determine the effects of semen quality on accessory sperm numbers and fertilization status/embryo quality in the artificially inseminated cow. All cows were bred conventionally with approximately 40 x 10^6 cells of either average or below average quality semen. The below average quality semen was the same semen described and used in experiment 1 (20% post-thaw motility, 74% primary abnormalities). The average quality semen used in this experiment possessed approximately 50% post-thaw progressive motility and 26% primary abnormalities (See Table 3). Immediately after insemination, cows were returned to pasture.

Embryo/ovum recovery and accessory sperm evaluations were performed according to procedures previously described.

Table 3. POST-THAW VIABILITY AND MORPHOLOGY CHARACTERISTICS OF CRYOPRESERVED SEMEN USED IN EXPERIMENT 2

Viability

Hours incubation at 37° C

Semen	Progressive Motility (Percent)			Intact Acrosomes (Percent)		
	0 h	3 h	6 h	0 h	3 h	6 h
Average	50	50	40	89.0	84.5	83.5
Below Average	20	20	10	71.0	66.0	62.5

Morphology

Semen	Normal	Abnormal Head Shapes					
		Taper	Crater	Asymmetric	Slightly Asymmetric	Long	Pyriform
Average	73	13	2	2	6	2	2
Below Average	26	16	8	9	25	14	2

(Values represent means of 3 replicates of 100 cells each)

Accessory Sperm Morphology

Embryos/ova recovered during Experiment 1 and embryos/ova recovered from cows bred with the low quality semen during Experiment 2 (all embryos/ova recovered from cows bred with below average quality semen) were combined for analysis of accessory sperm morphology. The objective was to determine to what extent morphologically abnormal spermatozoa could attach to and/or penetrate the zona pellucida of the ovum. Accessory sperm were evaluated morphologically under differential interference contrast microscopy at 1000x as described previously (See "Accessory Sperm Evaluation"). The proportion of abnormal cells in the inseminate was determined by procedures described in the section entitled "Post-Thaw Semen Evaluation". The inseminate was then compared with cumulative accessory sperm data for relative proportions of normal to abnormal cells using a studentized T-test.

Semen Preparation

Semen for experiments 1 and 2, was collected from several Holstein bulls with histories of either high (> 50%) or low (< 40%) percentages of morphologically abnormal cells. Ejaculates were collected via artificial vagina and subjectively evaluated for progressive motility. All ejaculates had at least 75% progressively motile cells immediately after collection. Ejaculates were pooled according to the bulls history for abnormal cells such that two populations of spermatozoa were separated for freezing. Four ejaculates from two bulls with a low percentage of abnormal cells were pooled and designated as "average quality semen". Nine ejaculates from four bulls with a high percentage of abnormal cells were pooled and designated as "below average quality semen". Spermatozoal concentrations of pooled ejaculates were then determined by spectrophotometric analysis.

The pooled ejaculates were extended and frozen in egg yolk citrate glycerol extender according to standard procedures of the artificial insemination industry. Prior to semen collection, the egg yolk citrate extender was prepared as follows:

	Fraction A	Fraction B
2.9% Sodium Citrate Dihydrate (w/v)	78%	66%
Egg Yolk (v/v)	20%	20%
Antibiotic (v/v)	2%	--
Glycerol (v/v)	--	14%

Prior to extension, the egg yolk citrate portion of the extender was clarified by syringe extrusion through a 1 um prefilter followed by a .45 um disposable filter assembly (both obtained from Gelman Sciences Inc., Ann Arbor, MI.) This step was not performed with the extender used in the Preliminary Experiment.

The extender antibiotics were a combination of Gentomicin (500 ug/ml), Tylosin (100 ug/ml) and Linco Spectin (300/600 ug/ml). This mixture was also added to raw semen immediately after collection at a rate of 20 ul/ml neat semen.

Aliquots of raw semen from each semen pool (average and below average) were then added to graduated cylinders containing an appropriate volume of Fraction A such that final volume of diluted semen would be sufficient to freeze approximately 200 straws at 40 x 10^6 cells per dose. Once extended in Fraction A, the diluted semen was poured into a plastic bag which was placed in a beaker of warm water (35°C) and transferred to a refrigerated environment (5°C) for cooling. Approximately 2 h later, Fraction B, which had previously been cooled to 5°C, was then added to Fraction A in 33 % (v/v) increments at 15 min intervals. The extended glycerolated semen was

loaded into .5 ml French straws which were held at 5°C until frozen in liquid nitrogen approximately 22 ± .75 h later.

Approximately two hundred straws of clarified extender were also frozen at this time for the purpose of further semen extension as described in the protocal for Experiment 1.

Post-Thaw Semen Evaluation

Cryopreserved semen used in this series of experiments was thawed according to procedures described previously (See "Conventional Artificial Insemination Procedures") and evaluated for spermatozoal viability and morphology. Three straws of frozen semen were removed from liquid nitrogen storage and plunged into a water bath at 35°C for 45 s. Straw contents were then pooled in a 1.5 ml Eppendorf tube and placed in a dry-bath incubator at 37°C. A slide was immediately prepared and observed at 100x using a phase-contrast microscope equipped with an air curtain set at 37°C for subjective evaluation of progressive sperm motility. Motility estimates were recorded to the nearest 10% at 0, 3, and 6 h of incubation at 37°C. The slide was then transferred to a differential interference contrast microscope and observed at 1000x magnification for evaluation of acrosomal integrity based on the prescence of the apical ridge. The percentage of intact acrosomes (PIA) was based on an average of two or three differential counts of 100 cells each (Saacke & Marshall, 1968). These procedures were then repeated to obtain values for progressive motility and percentage intact acrosomes after 0, 3, and 6 h of post-thaw incubation.

Morphology evaluations were conducted on a small aliquot of semen which was mixed approximately 1:1 with distilled water to inhibit sperm motility. This evaluation was performed under differential interference contrast microscopy at 1000x magnification. Only primary abnormalities

(abnormalities of the sperm head) were considered and were determined from the average percentage of three or four counts of 100 cells each.

Statistical Analysis

All statistical analyses were conducted with programs available through the Statistical Analysis System (SAS, 1985). Due to the high variation in accessory sperm numbers per ovum and the highly skewed nature of the population, several statistical procedures were performed on accessory sperm data prior to analysis. The data were first transformed by the following equation: $Log_{10}($ Value + 1). This step was performed to stabilize the variance in accessory sperm numbers per ovum and decrease the effects of extreme outliers within this population. Regression Diagnostic Influence was used to detect statistical outliers. Observations with R-student values greater than 3.5 were determined as statistical outliers. Transformed accessory sperm data were analyized for differences with respect to treatments and/or embryo quality by General Linear Models procedure and Fisher's Least Significant Differences.

Due to the subjective nature of embryo quality evaluations, embryos were grouped into catagories of excellent-good, fair-poor, and degenerates prior to statistical analysis. Chi-square contingency tables were used to detect changes in fertilization status/embryo quality distribution in response to various treatments and accessory sperm number. Correlations between these embryo/ovum quality groupings and the transformed accessory sperm data were conducted after assigning numerical values as follows: UFO = 1, Degenerate = 2, Fair-poor = 3, and Excellent-good = 4.

A studentized T-test was used to detect significant changes in the percentage of abnormal cells in the inseminate with proportion of abnormal cells recovered as accessory sperm.

Results

Preliminary Experiment

In this experiment, 26 embryos/ova were recovered from cows bred by conventional methods and 24 from cows bred with the blocking apparatus. The effects of retrograde sperm blockage at the time of insemination on accessory sperm parameters and fertilization rate are presented in Table 4. Blocking retrograde flow of sperm at the time of insemination had no significant effects on any of the parameters measured; however, blocking did cause a trend towards increasing accessory sperm numbers per embryo/ovum. A total of 341 accessory sperm were observed in the 26 embryos/ova recovered from control cows with a mean of 13.12 ± 28.85. The 24 embryos/ova recovered from cows bred with the blocking apparatus yielded a total of 485 accessory sperm with a mean of 20.21 ± 40.36 accessory sperm per ovum. Although these numbers were not statistically different, this trend suggested that accessory sperm numbers per embryo/ova could be increased by blocking retrograde flow at the time of insemination.

Table 4. PRELIMINARY EXPERIMENT: EFFECT OF CONVENTIONAL (CONTROL) AND BLOCKED INSEMINATIONS ON ACCESSORY SPERM (A.S.) PARAMETERS AND FERTILIZATION RATE

Treatment	n	Mean No. A.S. ± SD	Mean No. A.S. ± SD - Zeros * (n)	Median A.S. Value	Range A.S.	Percent Embryos/Ova with A.S.	Percent Fertilization
Control	26	13.1±28.8	18.9±33.3 (18)	4.5	0-141	62	81
Blocked	24	20.2±40.4	30.3±46.6 (16)	3	0-171	67	83

* Mean number accessory sperm per embryo/ovum for only those embryos/ova with accessory sperm.

On the contrary, median values of accessory sperm suggested that blocking interfered with sperm transport. The median values were 4.5 and 3 for embryos/ova in the control and blocked groups, respectively.

The standard deviations of accessory sperm numbers per embryo/ovum appeared to be proportional to the mean number accessory sperm per embryo/ovum for each of the respective treatment groups of the Preliminary Experiment. In each case, the standard deviation was approximately twice the mean of accessory sperm per embryo/ovum. This high variance in accessory sperm numbers per embryo/ovum undoubtedly caused problems in detecting statistical differences between the two populations. Figure 2 is a compilation of the data into histogram form showing the highly skewed nature of the population distribution relative to the percentage of embryos/ova that fall into various groupings of accessory sperm numbers for blocked and conventional inseminations, respectively.

Neither fertilization status nor embryo quality distribution were altered by blocking in this experiment.

The relationship between mean and median number of accessory sperm per embryo/ovum and fertilization status/embryo quality for all embryos/ova recovered during the Preliminary Experiment is presented in Table 5. There was an apparant increase in mean number of accessory sperm per embryo/ovum as fertilization status and/or embryo quality increased. Unfertilized ova had basically zero accessory sperm per ovum and were significantly different from the excellent-good and fair-poor embryo quality groupings ($P < .05$). Although not statistically different, excellent-good embryos tended to have higher accessory sperm numbers per embryo than the fair-poor grouping with means of 24.9 ± 43.3 and 13.9 ± 26.7, respectively. Degenerate embryos were not statistically different from any of the other quality groups but tended to be intermediate in accessory sperm number per embryo/ovum. The transformed accessory sperm data were significantly correlated with fertilization status/embryo quality in this experiment ($r = .5$, $P < .01$); suggesting that in-

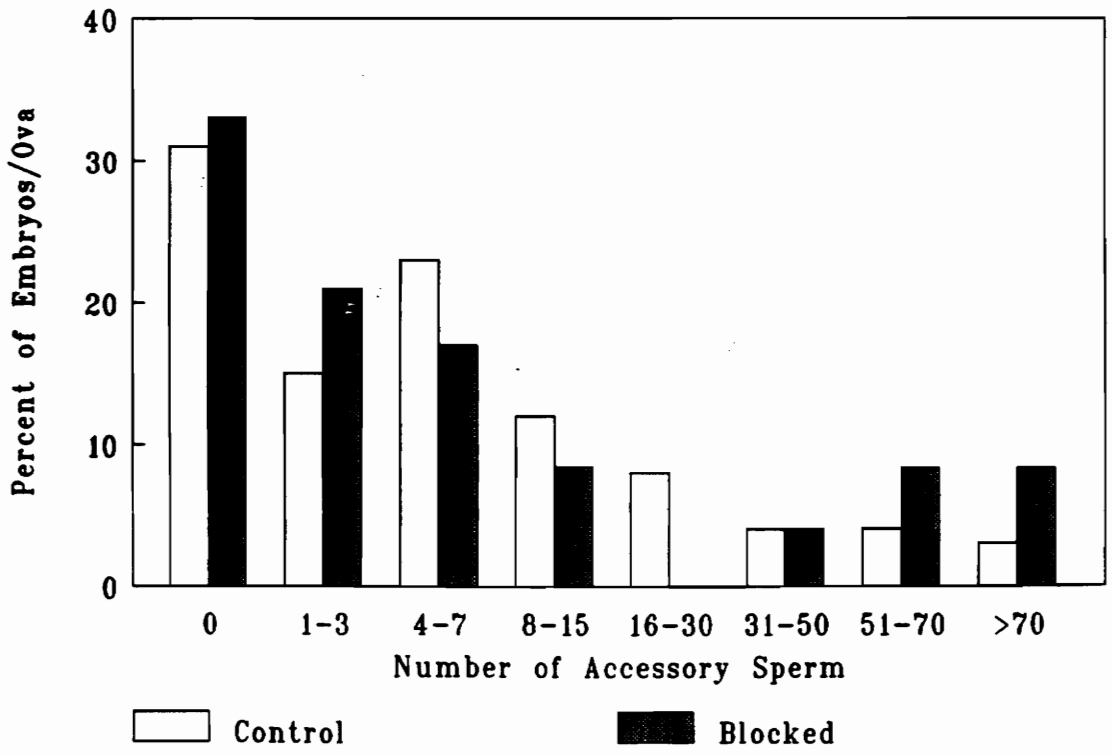

Figure 2. Preliminary Experiment: Effect of Conventional (Control) and Blocked Inseminations on Frequency Distribution of Embryos/Ova by Accessory Sperm Number

creasing accessory sperm numbers per embryo/ovum increased fertilization rates and/or embryo quality.

The median values for accessory sperm number tended to reflect the same trend as the mean values with regard to fertilization status/embryo quality. Excellent-good quality embryos had the highest median value of seven. Fair-poor and degenerate embryos were intermediate with value of three and four, respectively. Unfertilized ova had the lowest median with a value of zero.

When accessory sperm were evaluated in relation to fertilization status/embryo quality, it was interesting to note that the variance again appeared to be associated with the mean as it was when treatment effects were evaluated by accessory sperm number. Except for degenerate embryos, the standard deviation of accessory sperm number was approximately twice that of the mean value for each respective embryo quality grouping (Table 5).

Experiment 1

Although blocking did not significantly increase accessory sperm numbers per embryo/ovum in the Preliminary Experiment, the results were encouraging and we again tested the blocking apparatus in Experiment 1. Semen of poorer quality was selected for this experiment in an attempt to reduce the mean and associated variance in accessory sperm numbers per embryo/ovum. Also, effects of the blocking apparatus on accessory sperm numbers should have been more readily detected when marginal quality semen was used to inseminate. Sperm doses of 20 and 40 x 10^6 cells were used in a two by two factorial design with blocked and conventional inseminations. In view of the ease with which cows accepted the presence of the blocking apparatus during the Preliminary Experiment and the duration or retrograde flow (2 to 8 h, Mitchell et al., 1985), blocking time in Experiment 1 was increased to 3 h.

Table 5. PRELIMINARY EXPERIMENT: RELATIONSHIP BETWEEN ACCESSORY SPERM (A.S.) NUMBER AND FERTILIZATION STATUS/EMBRYO QUALITY

Fertilization Status/ Embryo Quality	n	Mean No. A.S. per Embryo/Ovum ± SD*	Median Value
EXCELLENT -GOOD	25	24.9 ± 43.3 a	7
FAIR-POOR	14	13.9 ± 26.7 a	3
DEGENERATE	2	4.0 ± 0.0 ab	4
UNFERTILIZED	9	.11 ± .33 b	0

* Values with different subscripts are significant at the alpha = .05 level

A total of 81 embryos/ova were recovered from the four treatment groups of Experiment 1. The effects of retrograde sperm blockage and sperm dosage on accessory sperm parameters fertilization rates are presented in Table 6. Despite the expected decreased mean and standard deviation of accessory sperm numbers in this experiment relative to the Preliminary Experiment, there was still no effect of retrograde blockage, semen dose nor their interaction on any of the parameters measured. Although the mean and variance in accessory sperm numbers per embryo/ovum decreased as expected, due to the utilization of poorer quality semen, the variance still appeared to be associated with the mean and again resulted in standard deviations approximately twice the mean value.

To evaluate the data for trends due to blockage, dosage data were pooled. The effects of retrograde sperm blockage per se on accessory sperm parameters and fertilization rates are presented in Table 7. There were no apparent trends of blocking on any of the parameters measured. The mean number accessory sperm per ovum ± standard deviation for blocked (n = 38) and control (n = 43) inseminations was 7.8 ± 14.9 and 9.4 ± 23.9, respectively.

To evaluate the results for trends due to sperm dosage, blocking data were pooled. The effects of sperm dose per se on accessory sperm parameters and fertilization rates are presented in Table 8. Although not significant, accessory sperm numbers per embryo/ovum showed a trend towards increasing with increasing sperm dosage. The mean number accessory sperm per embryo/ovum was 9.9 ± 17.1 (n = 39) and 7.7 ± 22.6 (n = 42) for the high (40 x 10^6 cells/dose) and low (20 x 10^6 cells/dose) sperm dosages, respectively.

There was no apparent shift in the relative percentage of embryos/ova which fell into various groupings of accessory sperm numbers as affected by blocking (Figure 3) or semen dose (Figure 4). Although the relative distribution was similar to that observed in the Preliminary Experiment (highly skewed), there was a slightly higher percentage of embryos/ova in the lower range of accessory sperm numbers. This was expected due to the utilization of poorer quality semen which resulted in a lower mean and associated variance of accessory sperm numbers per embryo/ovum. This is most apparent by comparing the median values for accessory sperm numbers between the

Table 6. EXPERIMENT 1: EFFECTS OF HIGH (40 X 10^6 CELLS) AND LOW (20 X 10^6 CELLS) SPERM DOSES AND CONVENTIONAL (CONTROL) AND BLOCKED INSEMINATIONS ON ACCESSORY SPERM (A.S.) PARAMETERS AND FERTILIZATION RATE

Semen Dose	Breeding Method	n	Mean No. A.S.±SD	Mean No. A.S.±SD -Zeros* (n)	Median A.S. Value	Range A.S.	Percent Embryos/Ova with A.S.	Percent Fertilization
High	Blocked	19	10.37±18.0	16.4±20.5 (12)	1	0-58	63	68
High	Control	20	9.45±16.7	17.2±19.6 (11)	1	0-55	55	65
Low	Blocked	19	5.21±11.0	14.1±14.7 (7)	0	0-42	39	55
Low	Control	23	9.45±29.1	14.5±35.4 (15)	1	0-141	65	74

* Mean number accessory sperm per embryo/ovum for only those embryos/ova with accessory sperm.

Table 7. EXPERIMENT 1: EFFECT OF CONVENTIONAL (CONTROL) AND BLOCKED INSEMINATIONS ON ACCESSORY SPERM (A.S.) PARAMETERS AND FERTILIZATION RATE

Treatment	n	Mean No. A.S.±SD	Mean No. A.S.±SD -Zeros * (n)	Median A.S. Value	Range A.S.	Percent Embryos/Ova with A.S.	Percent Fertilization
Control	43	9.4±23.9	15.6±29.3 (26)	1	0-141	60	72
Blocked	38	7.8±14.9	15.6±18.2 (19)	0	0-58	50	63

* Mean number accessory sperm per embryo/ovum for only those embryos/ova with accessory sperm.

Table 8. EXPERIMENT 1: EFFECT OF SPERM DOSE ON ACCESSORY SPERM (A.S.) PARAMETERS AND FERTILIZATION RATE

Sperm Dosage	n	Mean No. A.S.±SD	Mean No. A.S.±SD -Zeros * (n)	Median A.S. Value	Range A.S.	Percent Embryos/Ova with A.S.	Percent Fertilization
40 x 10^6	39	9.9±17.1	16.8±19.6 (23)	1	0-58	51	69
20 x 10^6	42	7.7±22.6	14.4±30.0 (22)	0	0-141	50	66

* Mean number accessory sperm per embryo/ovum for only those embryos/ova with accessory sperm.

Preliminary Experiment (3 and 4.5) and Experiment 1 (0 and 1) for blocked and conventional inseminations, respectively.

In this experiment, fertilization status/embryo quality was affected by method of insemination ($P < .1$). The effect was to increase the percentage of degenerate embryos and UFO at the expense of both fair-poor and excellent-good embryos (Figure 5). Semen dose had no significant effect on fertilization status/embryo quality in this experiment (Figure 6).

The relationship between mean and median number of accessory sperm per embryo/ovum and fertilization status/embryo quality for Experiment 1 is presented in Table 9. As in the Preliminary Experiment, the viable quality embryos (excellent-good and fair-poor embryo quality groups) had the highest mean number accessory sperm per embryo, but were not statistically different from each other. Degenerate embryos were again intermediate in accessory sperm number (4.3 ± 6.5) and the number of accessory sperm was significantly different from that of excellent-good (13.2 ± 17.4) and fair-poor (18.2 ± 34.2) embryo quality groups. Unfertilized ova, which most often had zero accessory sperm per ovum ($.23 \pm .65$), had significantly fewer accessory sperm than all other embryo quality groups. After transformation, accessory sperm data were again positively correlated with fertilization status/embryo quality ($r = .56$, $P < .01$).

As in the Preliminary Experiment, the median values for accessory sperm numbers per embryo/ovum followed the same trend as the mean value regarding fertilization status/embryo quality. Excellent-good quality embryos had the highest median value (5.5), fair-poor embryos were intermediate (4), while degenerate embryos and UFO both had median values of zero.

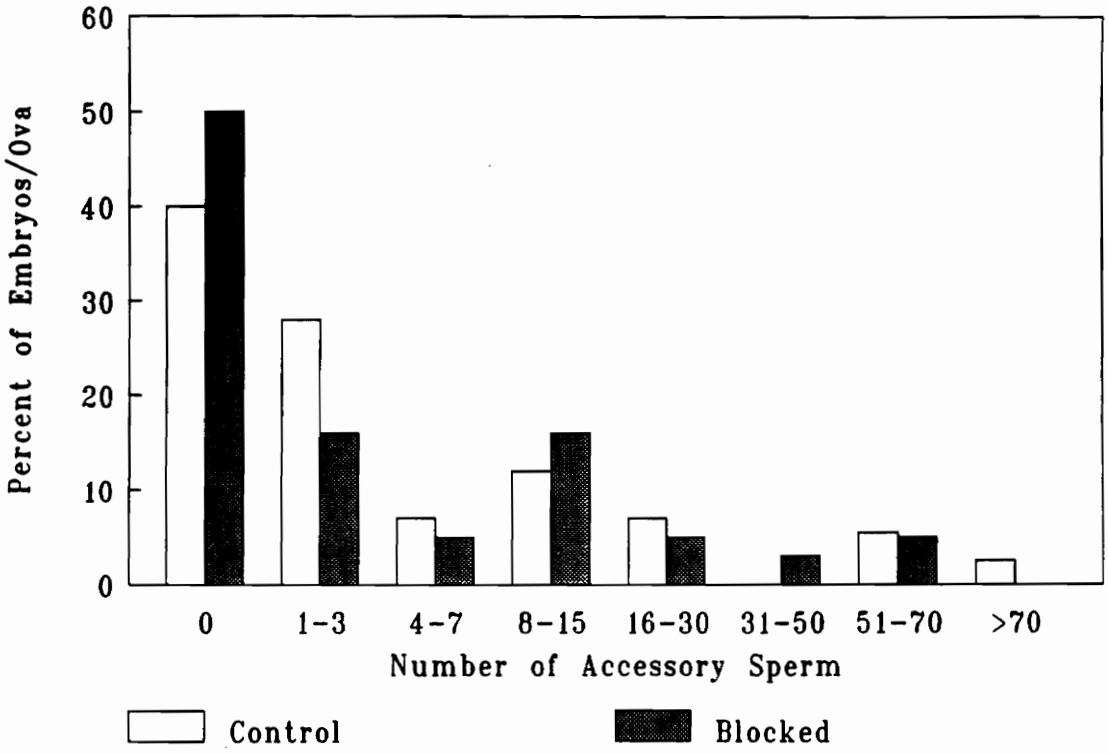

Figure 3. Experiment 1: Effect of Conventional (Control) and Blocked Inseminations on Frequency Distribution of Embryos/Ova by Accessory Sperm Number

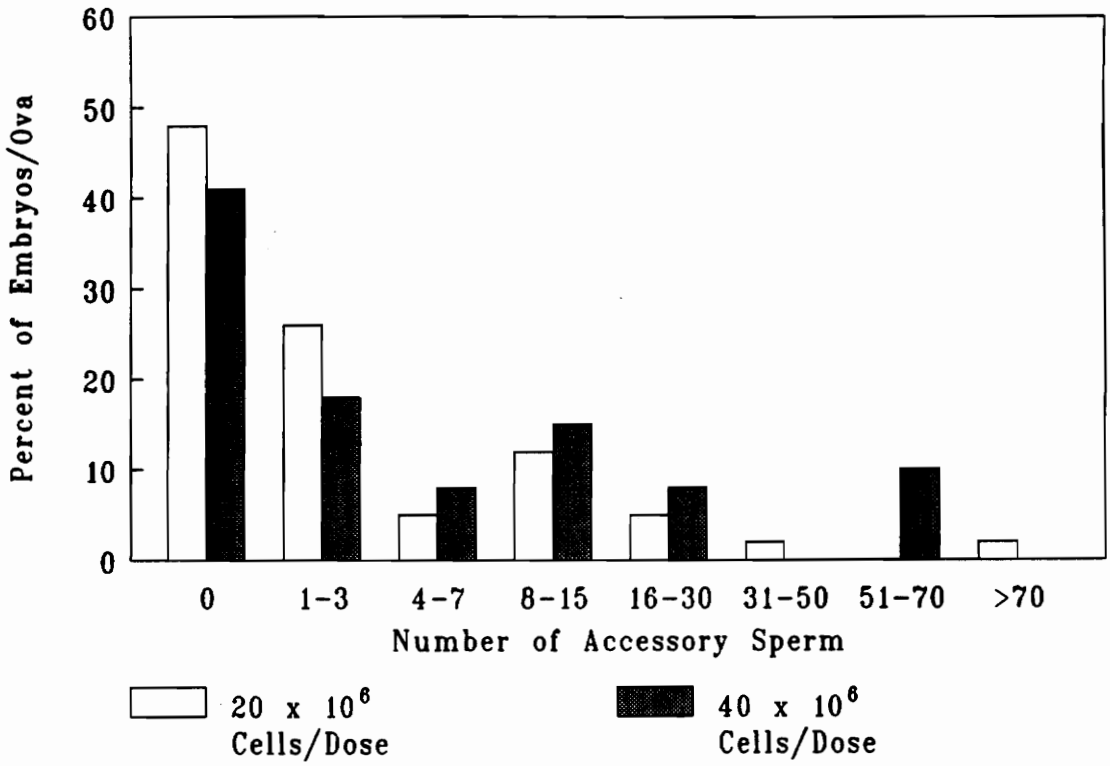

Figure 4. Experiment 1: Effect of Sperm Dosage on Frequency Distribution of Embryos/Ova by Accessory Sperm Number

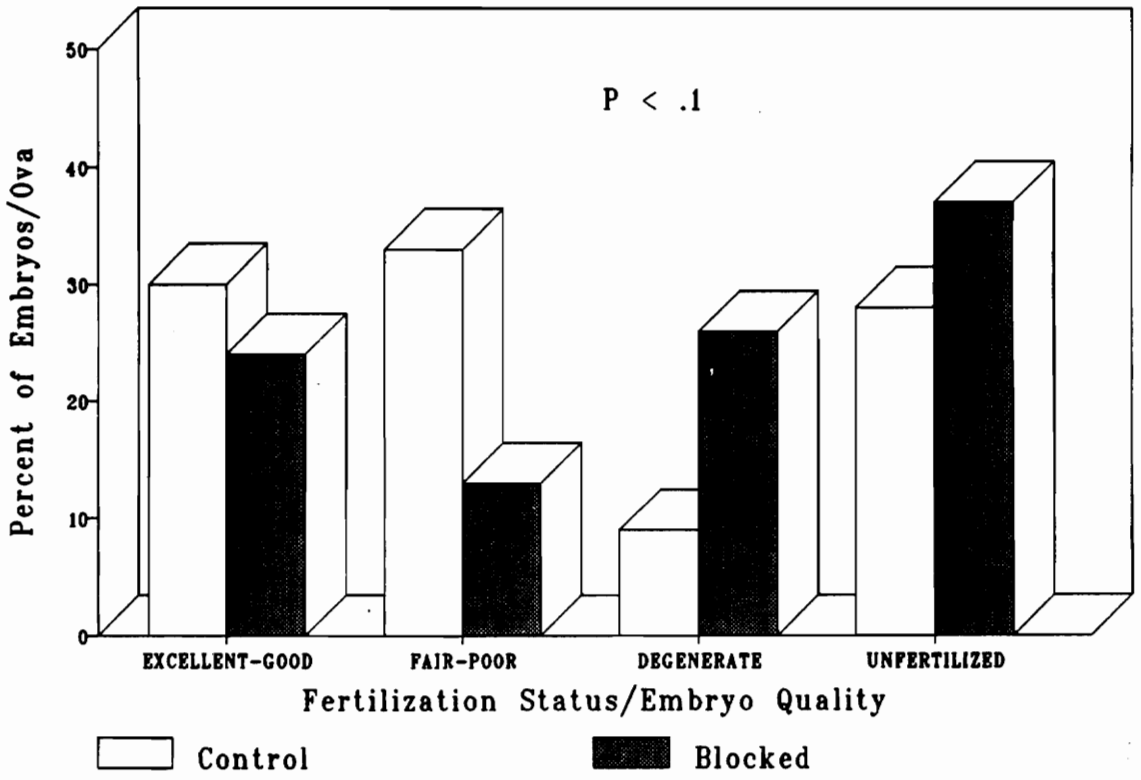

Figure 5. Experiment 1: Effect of Conventional (Control) and Blocked Inseminations on Fertilization Status/Embryo Quality

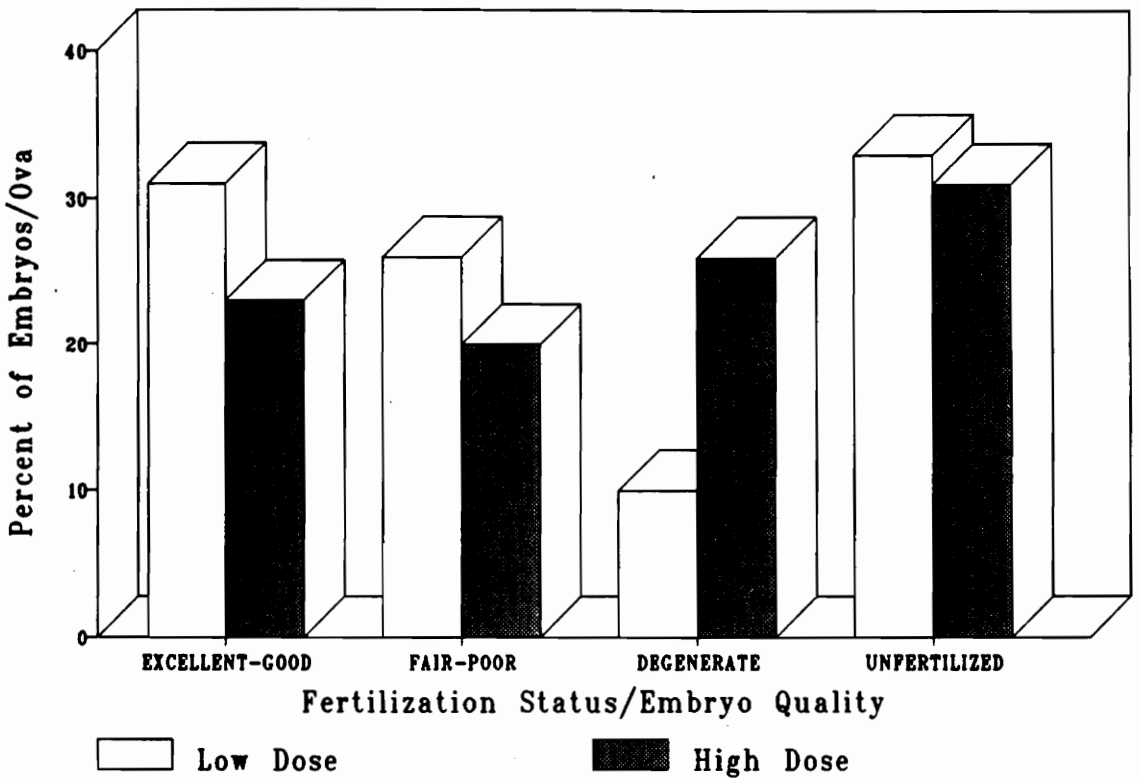

Figure 6. Experiment 1: Effect of Low (20 x 10^6 Cells) and High (40 x 10^6 Cells) Sperm Doses on Fertilization Status/Embryo Quality

Table 9. EXPERIMENT 1: RELATIONSHIP BETWEEN ACCESSORY SPERM (A.S.) NUMBER AND FERTILIZATION STATUS/EMBRYO QUALITY

Fertilization Status/ Embryo Quality	n	Mean No. A.S. per Embryo/Ovum ± SD *	Median Value
EXCELLENT -GOOD	22	13.2 ± 17.4 a	5.5
FAIR-POOR	19	18.2 ± 34.2 a	4
DEGENERATE	14	4.3 ± 6.5 b	0
UNFERTILIZED	26	.23 ± .65 c	0

* Values with different subscripts are significant at the alpha = .05 level

Experiment 2

The effects of semen quality (average vs below average) on accessory sperm parameters and fertilization rates are presented in Table 10. Semen quality had no significant effect on any of the parameters listed; however, there was a trend towards increasing fertilization status/embryo quality with average quality semen (Figure 7). Cows inseminated with average quality semen yielded 21% more excellent-good quality embryos and 27% fewer degenerates and UFO than cows inseminated with the below average quality semen. There was also an indication of a slightly greater fertilization rate using average quality semen as shown in Table 10.

The relationship between the mean and median accessory sperm number and fertilization status/embryo quality is presented in Table 11. The results are very similar to the previous two experiments with embryos classified excellent-good (11.5 ± 15.7) and fair-poor (13.4 ± 19.7) having the highest mean number accessory sperm per embryo, degenerate embryos (9.2 ± 15.2) being intermediate in number, and UFO (.57 ± 1.5) having the lowest mean with most containing zero accessory sperm per ovum. After transformation, the number of accessory sperm per embryo/ovum was again correlated with fertilization status/embryo quality ($r = .38$, $P < .05$).

The median values were not as clearly associated with fertilization status/embryo quality as in the previous experiments. In this comparison, fair-poor quality embryos had the highest median value (5) while excellent-good and degenerate embryos had similar intermediate values (3.5 and 3, respectively). The median value of 0 for UFO was consistant with previous experiments.

Table 10. EXPERIMENT 2: EFFECT OF AVERAGE AND BELOW AVERAGE QUALITY SEMEN ON ACCESSORY SPERM (A.S.) PARAMETERS AND FERTILIZATION RATE

Semen Quality	n	Mean No. A.S.±SD	Mean No. A.S.±SD -Zeros* (n)	Median A.S. Value	Range A.S.	Percent Embyos/Ova with A.S.	Percent Fertilization
Avgerage	21	8.4±14.9	11.7±16.5 (15)	2	0-58	71	90
Below Avgerage	22	11.3±16.2	15.5±17.2 (16)	3	0-48	68	77

* Mean number accessory sperm per embryo/ovum for only those embryos/ova with accessory sperm.

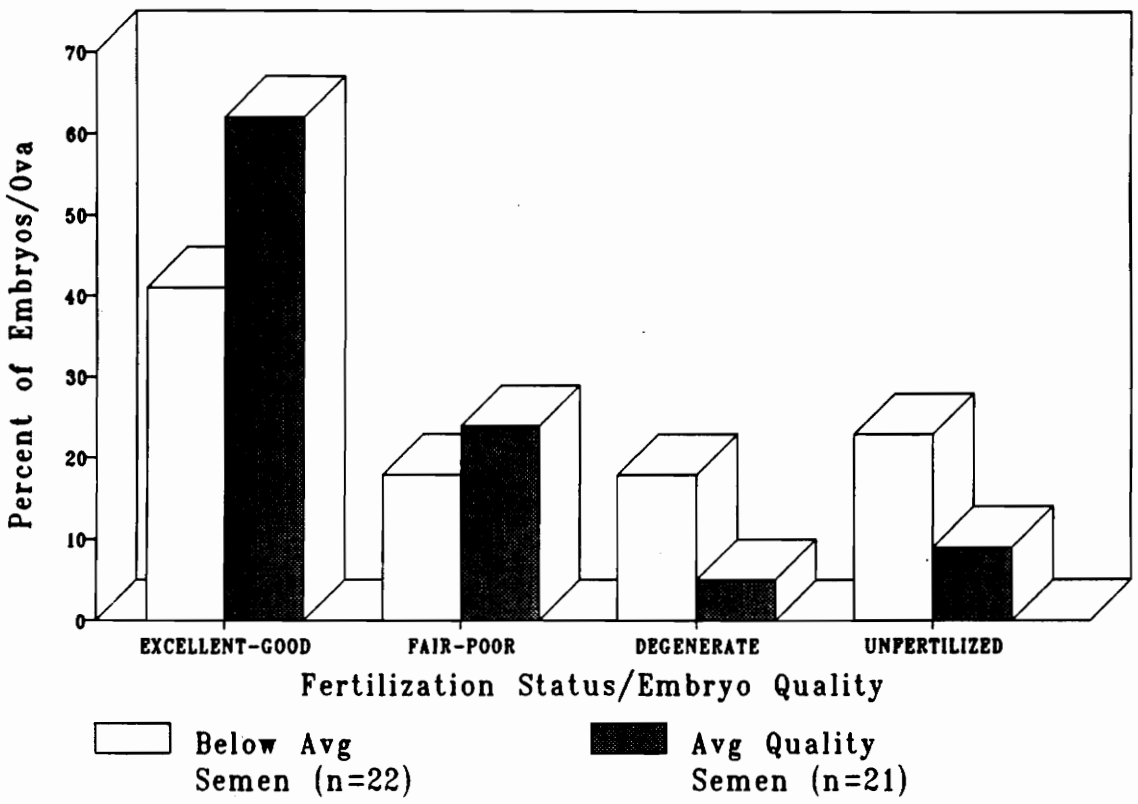

Figure 7. Experiment 2: Effect of Average and Below Average Quality Semen on Fertilization Status/Embryo Quality

Table 11. EXPERIMENT 2: RELATIONSHIP BETWEEN ACCESSORY SPERM (A.S.) NUMBER AND FERTILIZATION STATUS/EMBRYO QUALITY

Fertilization Status/ Embryo Quality	n	Mean No. A.S. per Embryo/Ovum ± SD *	Median Value
EXCELLENT -GOOD	22	11.5 ± 15.7 a	3.5
FAIR-POOR	9	13.4 ± 19.7 a	5
DEGENERATE	5	9.2 ± 15.2 ab	3
UNFERTILIZED	7	.57 ± 1.5 b	0

* Values with different subscripts are significant at the alpha = .05 level

Accessory Sperm Morphology Evaluations

Sixty-four embryos/ova with accessory sperm were recovered from cows bred with below average quality semen during experiments 1 & 2. These embryos/ova were evaluated for accessory sperm morphology and compared with the proportion of abnormal cells in the inseminate. Only primary abnormalities (abnormalities of the sperm head) were included in this evaluation. A total of 960 accessory sperm were counted and morphologically evaluated from the 64 embryos/ova. Evaluations revealed 513 accessory sperm (53%) to be normal and therefore, 447 (47%) to be abnormal in morphology. The proportion of abnormal cells found in 6 to 7 d embryos/ova (47%) was significantly different from the proportion of abnormal cells in the inseminate (74%, $P < .01$). Thus, there appeared to be a partial selection process within the sperm transport mechanisms or egg vestments to exclude morphologically abnormal spermatozoa.

The likelihood that any given abnormal spermatozoon will penetrate the zona pellucida of an ovum appears to be negatively correlated with the severity of the abnormality. This conclusion was based on the observation that, spermatozoa with slight or subtle abnormalites, such as craters and slight asymmetry, did not appear to be excluded, while more severe abnormalities (i.e. severely misshapen heads, asymmetrical) were critically excluded and virtually nonexistant as accessory sperm (Table 12).

Overall Results

The relationship between the mean and median accessory sperm number per embryo/ovum and fertilization status/embryo quality for 186 embryos/ova recovered across the three experiments is presented in Table 13. The embryo quality groups of excellent-good (16.4 ± 29.2) and fair-poor

Table 12. PROPORTION OF MORPHOLOGICALLY ABNORMAL ACCESSORY SPERM COMPARED WITH THE PERCENTAGE OF ABNORMAL SPERMATOZOA IN THE INSEMINATE

		Abnormal Head Shapes					
Source	Normal	Taper	Crater	Asymmetric	Slightly Asymmetric	Long	Pyriform
Accessory Sperm	53*	2.5	11	0	20	8	0
Inseminate	26*	16	8	9	25	14	2

* Columns values with astericks are significantly different ($P < .01$).

(15.8 ± 28.3) had the highest mean number accessory sperm per embryo/ova but were not statistically different from each other. Both excellent-good and fair-poor groups were significantly different ($P < .05$) in accessory sperm number from degenerate embryos which were intermediate in number with a mean of 5.4 ± 8.7. Unfertilized ova were significantly different from each of the other three groups with most containing zero accessory sperm per ovum (.27 ± .83, $P < .05$). The transformed accessory sperm data were significantly correlated with fertilization status/embryo quality ($r = .53$, $P < .01$) and suggested that increasing accessory sperm numbers would increase fertilization status/embryo quality.

The median values for accessory sperm number per embryo/ovum showed a distinct gradient with increasing fertilization status/embryo quality. The median value of zero accessory sperm for UFO was again consistant across the three experiments. Degenerate and fair-poor quality embryos were intermediate with median values of 1.5 and 3.5 accessory sperm, respectively. Excellent-good quality embryos had the highest median value of 5 accessory sperm.

The relationship among embryo quality, accessory sperm numbers and semen quality was further evaluated by grouping viable embryos, across all experiments, into two groups based on quality of semen used in the inseminate (average or below average). Each of these two groups of embryos were then divided into two subgroups based on embryo quality; high quality embryos (excellent & good) or low quality embryos (fair & poor). The relationship between embryo quality and accessory sperm numbers appeared to depend upon the quality of the spermatozoa in the inseminate. When average quality semen was used as the inseminate, embryos with greater than six accessory sperm had a significantly higher ratio of excellent-good to fair-poor quality embryos than embryos with less than or equal to six accessory sperm (Figure 8). Of the 21 embryos with greater than six accessory sperm 18 (86%) were of excellent-good quality and only 3 (14%) were of fair-poor quality. Thirty-six embryos with less than or equal to six accessory sperm were recovered from cows bred with average quality semen. Twenty of these embryos (56%) were of excellent-good quality and 16 (44%) were fair-poor quality embryos. This distribution was found to be significantly different at the $P = .05$ level.

Table 13. RELATIONSHIP BETWEEN ACCESSORY SPERM (A.S.) NUMBER AND FERTILIZATION STATUS/EMBRYO QUALITY - ACROSS ALL EXPERIMENTS

Fertilizatio Status/ Embryo Quality	n	Mean No. A.S. per Embryo/Ovum ± SD*	Median Value
EXCELLENT -GOOD	71	16.4 ± 29.2 a	5
FAIR-POOR	42	15.8 ± 28.6 a	3.5
DEGENERATE	22	5.4 ± 8.7 b	1.5
UNFERTILIZED	51	.27 ± .83 c	0

* Values with different subscripts are significant at the alpha = .05 level

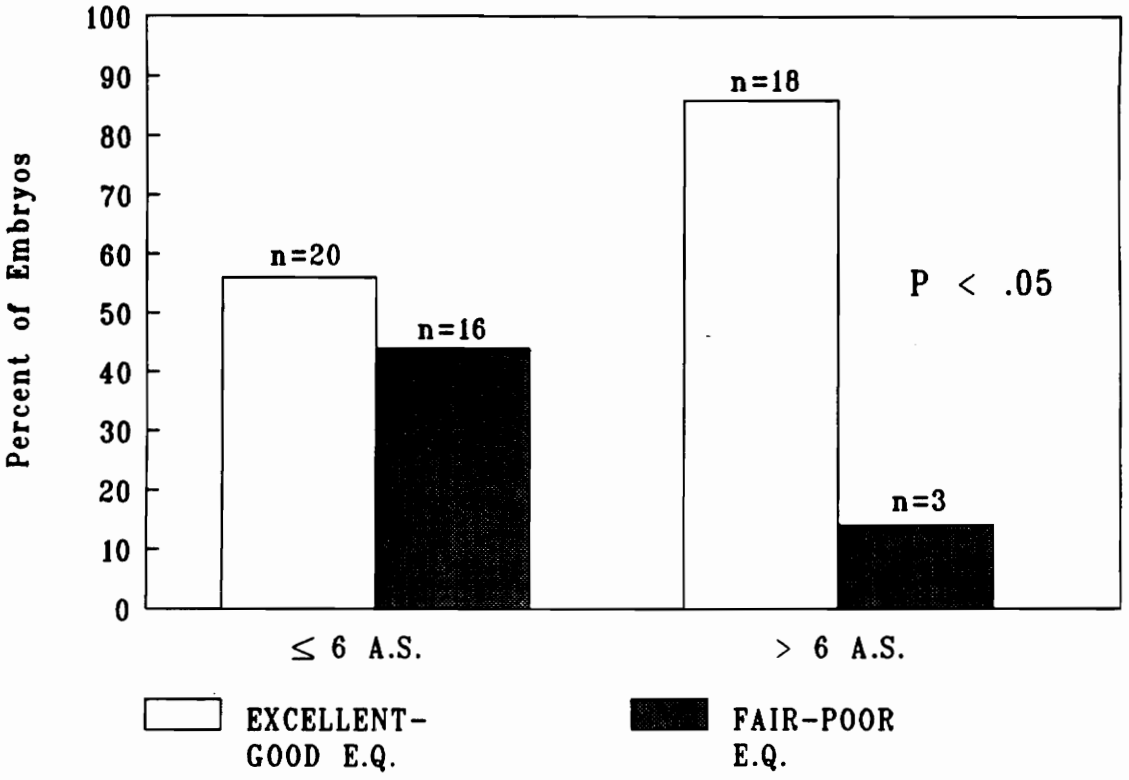

Figure 8. Relationship between Accessory Sperm Number (A.S.) and Embryo Quality (E.Q.) for Inseminations of Average Quality Semen

When below average quality semen was used to inseminate, there was no clear relationship between embryo quality and accessory sperm numbers (Figure 9). Twenty-five embryos with greater than six accessory sperm, yielded 14 (56%) excellent-good quality embryos and 11 (44%) fair-poor quality embryos. Twenty-nine embryos with less than or equal to six accessory sperm yielded 17 (59%) excellent-good quality and 12 (41%) fair-poor quality embryos. These results suggest that the relationship between embryo quality and accessory sperm numbers may be dependant upon semen quality.

The distribution, by accessory sperm number for 186 embryos/ova recovered across all experiments is shown in Figure 10. Note the highly skewed nature of the distribution. Although 82% of the embryos had 15 or less accessory sperm, the 18% in the tail to the right contain outliers which have marked effects on the mean number accessory sperm per embryo/ovum and extreme effects on the variances of this population. These high variances present significant problems in detecting statistical differences between populations (treatments) measured by accessory sperm number. Figure 11 shows the distribution of embryos alone (UFO excluded) by accessory sperm number. Although the curve shifted towards a more normal distribution, this is still a highly skewed population with a large proportion of embryos having zero (21.5%) or one to three accessory sperm (25.2%).

A total of 192 embryos/ova were recovered from 273 inseminations performed during this study for an overall recovery rate of 70%. There was no significant difference in recovery rate between experiments or between treatments within experiments. Six of the 192 embryos/ova were recovered as ruptured zonae pellucidae and were excluded from all statistical analysis due to inability to assign a reliable fertilization status and/or embryo quality. Another 12 embryos/ova were excluded from their respective experiments due to minor experimental complications (i.e. cow expelled the blocking apparatus prematurely, minor uterine infections, experimental error), but were included in the overall analysis of fertilization status/embryo quality with respect to semen quality and accessory sperm number.

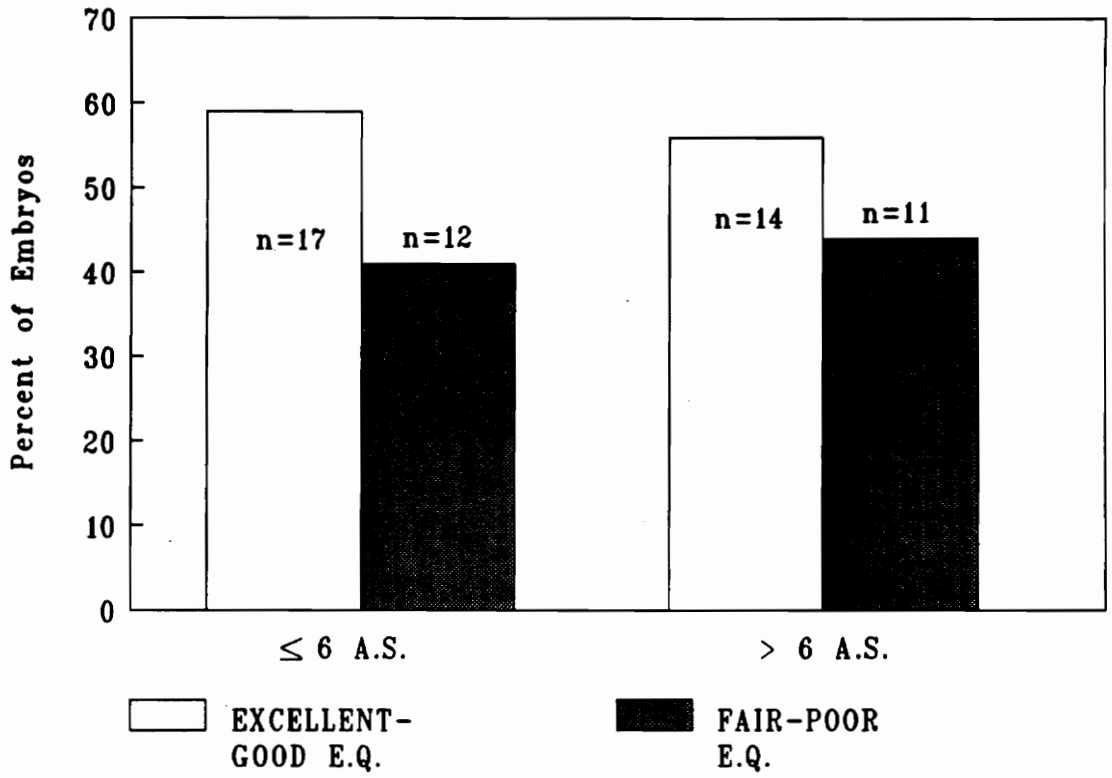

Figure 9. Relationship between Accessory Sperm Number (A.S.) and Embryo Quality (E.Q.) for Inseminations of Below Average Quality Semen

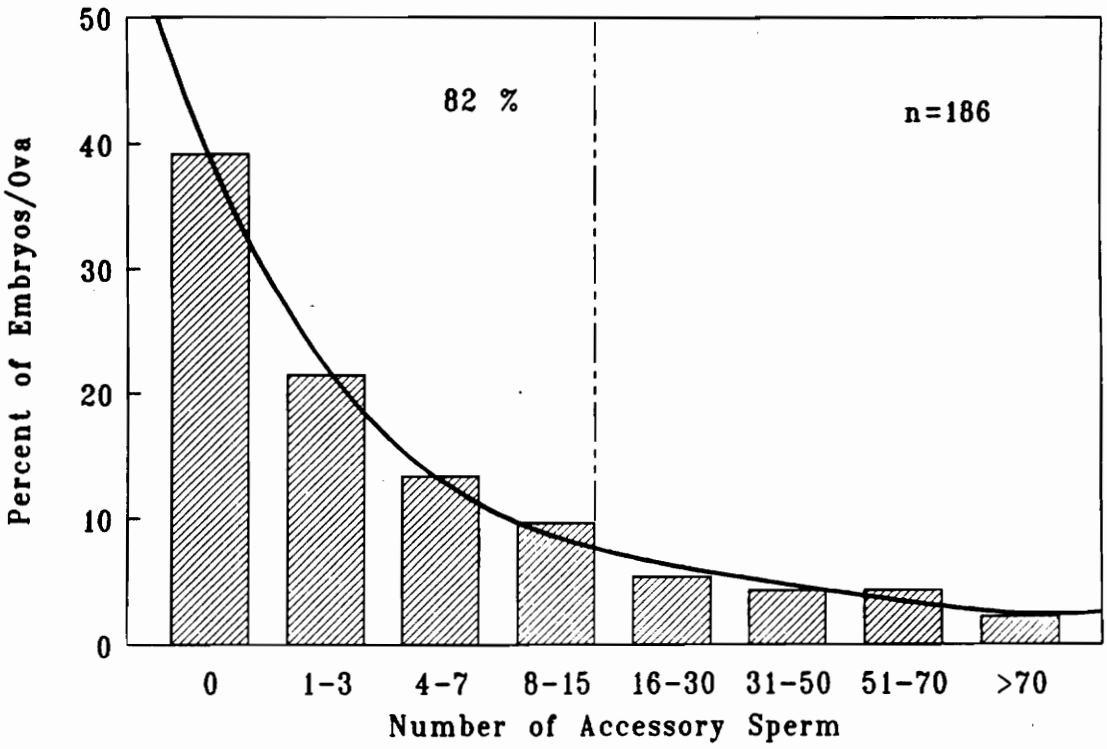

Figure 10. Frequency Distribution of Embryos/Ova by Accessory Sperm Number - Across All Experiments

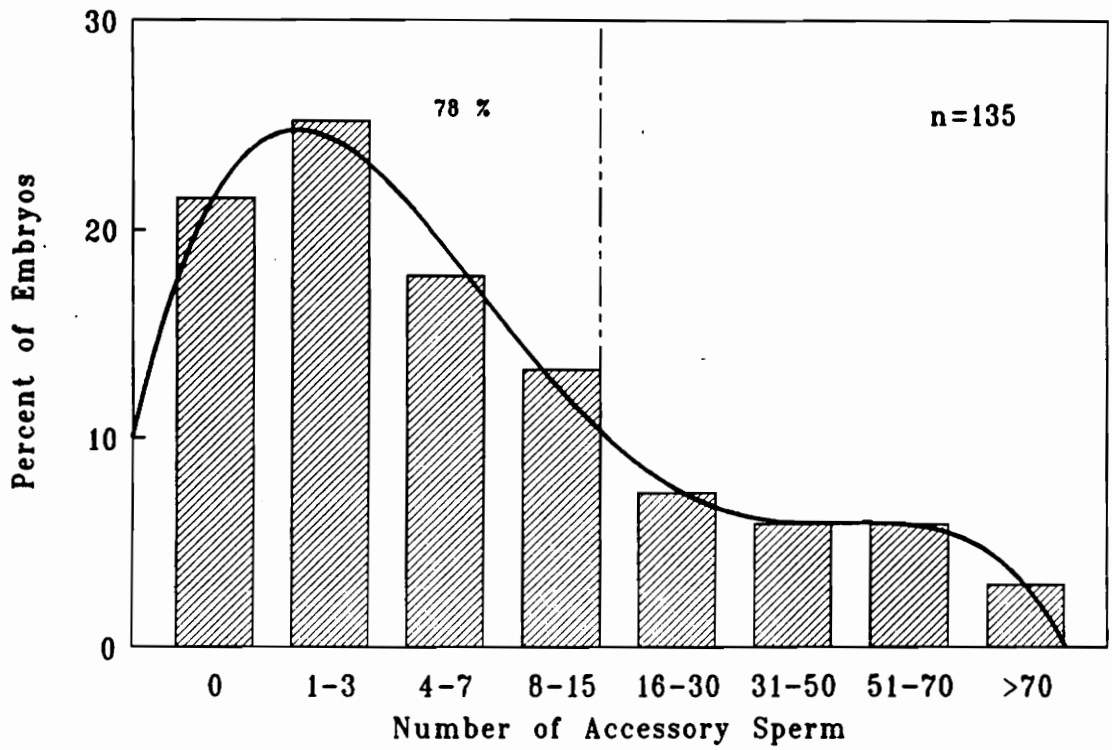

Figure 11. Frequency Distribution of Embryos by Accessory Sperm Number - Across All Experiments

Cows in this study were observed to ovulate from the right ovary more frequently than the left. Via rectal palpation of the 273 cows flushed, 151 ovulations (55%) were observed to occur on the right ovary while 122 (45%) occurred from the left ovary and was significantly different at the P = .05 level.

Discussion

Retrograde Sperm Blockage

Blocking retrograde flow at the time of insemination did not affect accessory sperm numbers per embryo/ovum. However, in the Preliminary Experiment, blocking retrograde flow for 1 h showed a trend towards increasing accessory sperm numbers per embryo/ovum. Undoubtedly, the values were not statistically significant due to the extremely high variance associated with accessory sperm numbers (Table 4 on page 39). Embryos/ova recovered from conventional inseminations averaged 13.1 ± 28.9 accessory sperm while embryos/ova recovered from blocked inseminations had a mean value of 20.2 ± 40.4. This difference was even more apparent, but still not different ($P > .1$), when only embryos/ova having accessory sperm were evaluated for mean number of accessory sperm per embryo/ovum (18.9 ± 33.3 for controls and 30.3 ± 46.6 for blocked). Although not significant, these results were sufficently encouraging to pursue the same objectives in Experiment 1.

A blocking time of only 1 h following insemination was used during the Preliminary Experiment because it was unknown how cows would react to the blocking apparatus. Since no adverse effects or excessive restlessness were observed among cows during this blocking interval and the fact that

the duration of retrograde loss following insemination was shown to be approximately 6 to 8 h (Mitchell et al., 1985; Nelson et al., 1987), blocking time during Experiment 1 was increased to 3 h. In Experiment 1, semen quality was lower than that used during the Preliminary Experiment. The lower quality semen was selected under the assumption that any effects of the blocking apparatus on sperm retention and ultimately accessory sperm numbers would be more readily detectable with semen of marginal quality. This rationale was based on the positive association of the mean with the variance in accessory sperm numbers; i.e. a reduced mean accessory sperm number is accompanied by a reduced variance.

In Experiment 1, blocking for 3 h following insemination showed neither a significant effect nor a trend in relation to accessory sperm numbers per embryo/ovum, despite the expected decrease in accessory sperm mean and decreased variance in relation to that observed in the Preliminary Experiment. Although neither experiment revealed a significant increase in accessory sperm numbers in response to retrograde blockage, it is apparant that blocking had no adverse effects on sperm transport that were detectable as measured by accessory sperm number (Table 7 on page 46).

Whether or not sperm retention was actually improved by this blocking procedure is unknown. It is possible that sperm retention was significantly increased during these experiments; but, our indirect measure of this retention by accessory sperm numbers may not have been sensitive enough to detect the change. Thus, we may only have seen a trend towards increased accessory sperm numbers such as in our Preliminary Experiment.

Hawk and Conley (1971) undoubtedly increased sperm retention by ligating ewe tracts at the vulvo-vaginal junction following insemination. At slaughter, 24 h later, 62% of the total inseminate could be recovered from the reproductive tract of ewes which had been ligated while only 1% of the inseminate could be recovered from unligated tracts. Despite this apparent increase in sperm retention, fewer sperm were actually found in the oviducts of the ligated tracts when compared to controls. The authors suggested that ligature itself had caused somewhat of an I.U.D. effect on sperm transport. They compared these results with those of Hawk (1970) and Conley and Hawk

(1970) where insertion of a plastic spiral into the uterine body of sheep caused myometrial contractions to be propagated towards the cervix rather than towards the oviducts and thereby totally inhibited sperm transport to the oviducts.

Based on experience with excised tracts using the breeding apparatus, it is not unreasonable to suspect that the blocking apparatus used in this study did increase sperm retention; thus, the trend of increased accessory sperm numbers observed in the Preliminary Experiment is believable. It is also not unreasonable to suspect the blocking apparatus, if allowed to remain in place for extended periods of time (3 h vs. 1 h), could exert an I.U.D. effect and alter sperm transport thereby negating the effect of increased sperm retention. Thus, the descrepancy between the Preliminary Experiment, which resulted in a trend towards increasing accessory sperm numbers, and Experiment 1 in which there was no effect of blocking on accessory sperm numbers could be the result of blocking time. If in fact the blocking apparatus did have an I.U.D. effect on sperm transport, it was not as severe as that described by Hawk or Conley and Hawk since there was no negative effect on fertilization rate, percentage of embryos/ova with accessory sperm nor on mean number accessory sperm per embryo/ovum.

On the other hand, the median number of accessory sperm for the Preliminary Experiment and Experiment 1 was depressed for the blocked treatment; suggesting, that blocking had a negative effect on accessory sperm numbers and/or sperm transport. Due to the highly skewed nature of this population, the median may be the more important value regarding accessory sperm.

Although there was a slight trend towards increased accessory sperm numbers by blocking in the Preliminary Experiment, there was no evidence of a corresponding shift in fertilization status/embryo quality. This is in conflict with data and research of other investigators (Hunter and Wilmut, 1984; Weitze et al., 1988; Saacke et al., 1988) that suggested accessory sperm numbers were correlated with fertility; however, the number of embryos/ova obtained during this experiment is probably insufficient to detect significant changes in these parameters.

In Experiment 1, blocking retrograde flow at the time of insemination apparantly had a negative effect on fertilization status/embryo quality collectively (Figure 5 on page 51), despite the fact that accessory sperm numbers were not effected. Blocking, in this experiment, resulted in a higher proportion of UFO/degenerate embryos in relation to all embryos/ova when compared with conventional inseminations. This may be the result of an I.U.D. effect of the blocking apparatus, not on sperm transport per se, but on the uterine/oviductal environments. Such an effect could have been initiated by the release of prostaglandin from an irritated endometrium which may set in motion a complex series of hormonal and biochemical reactions adversely affecting the uterus/oviduct and/or their ability to sustain embryonic development.

Sperm Dosage

Accessory sperm numbers per embryo/ovum showed an insignificant trend towards increasing with the higher sperm dosage (Table 8 on page 47). This was an unexpected result; but, not surprising when we consider that we are inseminating cows with sperm doses of 20 to 40 million cells and measuring responses usually at less than 20 accessory sperm per embryo/ovum. It appears that sperm access to the oviduct is a sensitive process that can shift from zero sperm to thousands of sperm reaching the site of fertilization at a given time (Mattner 1968; El-Banna and Hafez, 1970) in response to minor changes in the breeding or fertilization environment (sperm dose, semen quality, breeding technique, semen handling, stage of estrus, etc.). If millions of sperm are inseminated and sperm transport mechanisms are sufficient to allow one sperm access to the oviduct, then why shouldn't there be at least hundreds if not thousands of other spermatozoa capable of competing to be the fertilizing spermatozoon and/or to become an accessory sperm when one considers all the variables and their respective effects?

Weitze and coworkers (1988) observed that accessory sperm numbers in pig embryos increased in response to increasing numbers of sperm in the inseminate. Pigs, however, being multiple ovulators, are more capable of diluting the effects of the sperm transport system by spreading the numbers of sperm at the site of fertilization across a dozen or more embryos/ova. In a single ovulating species there is only one embryo/ovum from which to gain accessory sperm data and make evaluations regarding sperm transport and/or treatment effects. Thus, with a greater number of embryos/ova to generate accessory sperm data, each individual embryo/ovum becomes less important and more reliable values for numbers of sperm at the site of fertilization are likely to be obtained.

Although there was a slight but insignificant shift in accessory sperm numbers in response to sperm dose (Experiment 1), there was no corresponding shift or trend in fertilization status/embryo quality (Figure 6 on page 52); In fact it was to the contrary. Although the distributions were not statistically different, the high sperm dose tended to yield a higher proportion of degenerate embryos and lower proportion of both excellent-good and fair-poor embryos than did the lower sperm dose. The percentage of UFO (fertilization rate) was similar for both doses. Considering that marginal quality semen was used for this experiment, the results tended to conflict with those of Sullivan and Elliott (1968) who observed that low fertility bulls had the greatest reponse in fertility to increases in sperm concentration of the inseminate. However, it is likely that the number of embryos/ova recovered during this experment was insufficient to detect statistical differences in these parameters.

Semen Quality

Fertilization status/embryo quality tended to be higher in embryos recovered from cows bred with average quality semen compared to below average quality semen (Experiment 2). Although the shift was not statistically significant, average quality semen yielded 21% more excellent-good

quality embryos and 27% fewer degenerates and UFO than did the below average quality semen (Figure 7 on page 56). These data are similar to those reported by Miller and coworkers (1982). In their study, a bull with a high percentage of cratered sperm was used to breed superovulated cows. Cows served by semen from this bull yielded a lower proportion of fertilized embryos and a higher proportion of non-transferrable embryos than cows bred with semen from control bulls. Our data and those of Miller suggest a male-related factor may be partially responsible for early embryonic mortality. Hillery and coworkers (1990) have presented evidence from in vitro inseminations which also suggest that bulls of different fertility levels differ in their ability to sustain embryonic development following fertilization. In their study, there was no difference between the two groups of bulls (high and low fertility) in fertilization rates; however, the percentage of cleaved oocytes/zygotes that developed to the compacted morula blastocyst stage was significantly higher for the bulls in the high fertility group.

Results similar to those of Hillery and coworkers were observed by Eyestone and First (1989). In that study, semen from four bulls was evaluated for its ability to fertilize oocytes and sustain embryonic development, in vitro. There was no difference in fertilization rates among bulls; however, a difference was observed among bulls in their ability to sustain embryonic development to the late morula or early blastocyst stage.

With respect to embryonic mortality, the results of this research and the in vitro studies of Eyestone and First (1989) and Hillery and coworkers (1990) tend to agree with the in vivo studies of Bearden and coworkers (1956) and Kidder and coworkers (1954) who evaluated that the major factors responsible for returns to estrus (fertilization failure and early embryonic death) for high and low fertility bulls. They found the major factor responsible for returns to estrus for low fertility bulls was fertilization failure; however, embryonic death seemed to be the principle cause of returns to estrus for the high fertility bulls which approached 100% fertilization rates. Although embryonic death was most often responsible for returns to estrus in the high fertility bulls, the relative percentage of returns to estrus due to embryonic death was higher for the low fertility bulls.

Collectively these studies present evidence which strongly implicate the male as having a direct effect on early embryonic mortality. If so, it is not only important that an ejaculate or dose of semen be characterized by good motility and viability in order to transverse the barriers of the female tract, thus gaining access to the oviduct and ultimately fertilize the ovum; there is apparently another measure of semen quality that must be considered which relates to the ability of sperm in that ejaculate or dose to be sufficiently competent to sustain embryonic development once fertilization has taken place. This component may be the uncompensible factor that will not respond to increased sperm doses with increased fertilization rates (Saacke et al., 1988b).

Semen quality apparently had an effect on differences in accessory sperm numbers per embryo/ovum between our Preliminary Experiment and Experiment 1. The Preliminary Experiment utilized semen considered to be of average quality while Experiment 1 used a lower or below average quality semen. The overall means for number of accessory sperm per embryo/ovum were 16.5 ± 34.7 and 8.7 ± 20.1 for the Preliminary and Experiment 1, respectively. This shift in accessory sperm numbers is also apparent when the median values of accessory sperm for the respective experiments are examined. The median values for blocked and control inseminations in the Preliminary Experiment were 3 and 4.5, respectively (Table 4 on page 39). Despite the fact that sperm doses in Experiment 1 was either equal to or twice that of the Preliminary Experiment, the median values across dosages decreased to 0 and 1 for blocked and control inseminations, respectively (Table 7 on page 46). Because these experiments are confounded over time, these values were not analyzed for statistical significance. It is interesting to note; however, that the Preliminary Experiment, which had the higher mean and median values, was conducted during the months of May thru August 1989. Due to the warmer environmental temperatures during this period, one would usually expect to see a decrease in fertility when compared to animals, like those of Experiment 1, which were bred during the cooler months of the year (fall, winter or spring; 1989-90).

Although fertilization status/embryo quality was slightly shifted in favor of average quality semen in Experiment 2, semen quality did not significantly alter the mean number accessory sperm per embryo/ovum. In fact, the mean number accessory sperm per embryo/ovum was shifted slightly

in favor of the lower quality semen with means of 8.4 ± 14.9 and 11.3 ± 16.2 for average and below average quality semen, respectively (Table 10 on page 55). These data conflict with that of Saacke and coworkers (1988b) and Weitze and coworkers (1988) in which increased accessory sperm numbers per embryo/ovum resulted in a trend towards increased fertilization and embryo survival rates. However, these experiments were conducted utilizing semen of reasonably consistant quality. Thus, data from this experiment may suggest that the relationship between embryo quality and accessory sperm numbers may be dependant upon the quality of semen used to inseminate. This concept becomes more apparent when we separate embryos recovered across all experiments by quality of inseminate and evaluate embryo quality responses to accessory sperm numbers. When average quality semen was used as the inseminate, embryos with six or less accessory sperm resulted in a near 50:50 ratio of excellent-good to fair-poor quality embryos (Figure 8 on page 62). This ratio was significantly shifted to 86% excellent-good and 14% fair-poor when accessory sperm numbers per embryo were greater than six and average quality semen was used as the inseminate ($P < .05$). When below average quality semen was used to inseminate, there was no apparent breaking point in accessory sperm numbers and the resulting embryo quality distributions were 55 to 60% excellent-good and 40 to 45% fair-poor regardless of accessory sperm number (Figure 9 on page 64). There is no significance to the number six when evaluating accessory sperm numbers. Six just happened to be the point at which embryo quality distribution shifted for average quality semen in this data set. However, this data set is relatively small and, with a greater number of embryos, the actual distribution may be different than that observed in this series of experiments.

Results of these experiments also tend to support the theory of compensible and uncompensible fertility components related to semen quality (Sullivan and Elliott, 1968; Saacke et al., 1988b). Average quality semen yielded a slightly higher proportion of excellent-good quality embryos in response to increasing accessory sperm numbers. With below average quality semen, it appears that, regardless of accessory sperm number, incompetent spermatozoa fertilize oocytes with equal frequency to competent sperm; thus, resulting in a higher proportion of degenerate and low quality embryos when compared with embryos recovered from cows bred with average or better quality

semen. These results should not be interpreted to suggest that incompetent spermatozoa from below average quality semen are more competitive in the fertilization process than incompetent spermatozoa from average quality inseminates. Rather, it would seem more logical to consider that ejaculates or inseminates with high proportions of morphologically abnormal spermatozoa would contain higher proportions of incompetent spermatozoa when compared with ejaculates or inseminates that contain a high percentage of morphologically normal cells. Thus, the resulting effect of semen quality on embryo quality would be due to differences in relative numbers or ratios and not per se to the competitiveness of competent and incompetent spermatozoa from different ejaculates. Ejaculates containing a high proportion of incompetent spermatozoa would therefore be likely to result in an increased rate of early embryonic mortality that could not be compensated for by increasing numbers of sperm in the inseminate dose.

Accessory Sperm vs. Fertilization Status/Embryo Quality

Within and across all experiments of this study, there appeared to be a trend towards increasing fertilization status/embryo quality with increasing accessory sperm numbers (Table 5 on page 43, Table 9 on page 53, Table 11 on page 57, Table 13 on page 61). In each case, viable quality embryos (excellent-good and fair-poor) had the highest mean for accessory sperm numbers per embryo/ovum. Degenerate embryos were intermediate while most UFO had zero accessory sperm per ovum. When embryos/ova were combined and evaluated across all experiments, the number of accessory sperm present in viable quality embryos were significantly greater than that observed in degenerate embryos, which appeared to be intermediate in accessory sperm number. Most UFO possessed no accessory sperm per ovum and the mean number was significantly lower than all other embryo quality groups.

The median values varied to some degree within experiments, but showed the same trend as the means with viable quality embryos having the higher values and UFO consistantly having a median of zero. Combining embryos/ova across all experiments, a distinct gradient in the median value for accessory sperm number was observed as fertilization status/embryo quality increased. Unfertilized ova had a median value of 0; degenerates, 1.5; fair-poor, 3.5; and excellent-good, 5 (Table 13 on page 61).

These results are consistant with the research of Saacke and coworkers (1986), where a similar relationship was observed between accessory sperm numbers and fertilization status/embryo quality in superovulated cows. In their study, the mean numbers of accessory sperm per embryo/ovum were considerably lower for each respective catagory/quality; however, accessory sperm did appear to increase in numbers with increasing fertilization status/embryo quality.

The results of these studies suggest that by increasing accessory sperm numbers per embryo/ovum, fertilization rates can be increased and early embryonic deaths reduced by producing better quality embryos. If in fact this is the case, our thoughts for future research should be directed to determine: 1. What are the factors controlling or regulating the numbers of accessory sperm per embryo/ovum and can these factors be controlled or overriden? 2. How high can accessory sperm numbers per embryo/ovum be raised? 3. Does this trend towards increased embryo quality continue at higher accessory sperm numbers or is there an optimum value past which embryo quality does not respond to increasing accessory sperm numbers.

Accessory Sperm Morphology

The results of accessory sperm morphology evaluations suggest that there is a partial selection process within the sperm transport mechanisms of the female reproductive tract and/or the zona

pellucida of the ovum. The inseminate used in this study contained approximately 74% morphologically abnormal cells. Cumulatively, accessory sperm in embryos/ova recovered from these inseminations were observed to have only 47% morphologically abnormal cells. These values were significantly different at the P = .01 level (Table 12 on page 59).

Although 47% of the accessory sperm recovered from these embryos/ova were abnormal in morphology, the abnormalities observed in accessory sperm were quite subtle compared to those of the inseminate. The mode catagory of abnormal sperm morphology was "slightly asymmetrical". This catagory was defined as spermatozoa with only slight or very subtle irregularities or distortions of the sperm head. Morphological evaluations of accessory sperm and the inseminate were extremely critical during this study and many of the spermatozoa which were determined to be slightly asymmetrical would probably have been classified as normal cells in routine evaluations. Spermatozoa with severe morphological abnormalities (asymmetrical, pyriform, misshapen heads, etc.) were almost completely excluded from the accessory sperm population.

These results are consistant with those of Saacke and coworkers (1988a). Although their inseminate contained a higher proportion of normal cells (61%) than the inseminate used in this study (26%), Saacke observed an enrichment of accessory sperm with morphologically normal cells (75.5%). The types of abnormal accessory sperm observed by Saacke were also similar to those found in our study. There appeared to be little or no selection against cratered spermatozoa that were otherwise normal in shape. Tapered sperm appeared to be partially selected or excluded and severely misshapen spermatozoa were almost totally excluded from the accessory sperm populations. These studies suggest that morphologically abnormal spermatozoa are selected against during the sperm transport and/or fertilization process in the female tract. The intensity of this selection process appears to be correlated with the severity of the abnormality in question.

Exactly where and when this selection process occurs in the female reproductive tract is a question that has been under considerable investigation. Recent studies have shown that spermatozoa with abnormal head shapes have been excluded at the cervix (Mortimer, 1977; Karabinus & Saacke,

1987), uterus (Mitchell et al., 1985), and the uterotubal junction (Krzanowska, 1974). In Krzanowska's study (1974), rat spermatozoa with severe morphological abnormalities were excluded at the uterotubal junction but those with subtle abnormalities reached the site of fertilization readily and were found capable of fertilization in vitro (Krzanowska & Lorenc, 1983). This agrees with the results observed in this study in that severely abnormal spermatozoa were not recovered as accessory sperm while those with subtle abnormalities were not excluded and thus were recovered as accessory sperm.

It is also possible that morphologically abnormal spermatozoa select against themselves in the sperm transport process. Dresdner and Katz (1981) observed that spermatozoa with abnormal head shapes displayed an altered motility pattern which may be responsible for a decreased effeciency of sperm transport.

Accessory Sperm Distribution

Regardless of experiment or treatment, accessory sperm numbers appeared to follow the same general distribution pattern throughout the study. In each case, the mode value for accessory sperm numbers per embryo/ovum was zero and the proportion of embryos/ova within a particular value decreased as the accessory sperm number increased (Figure 10 on page 65). This resulted in a highly skewed population with the majority of embryos/ova represented by the lower range of accessory sperm values. However, the embryos/ova located in the tail of the distribution (with the higher accessory sperm values) had extreme effects on the variances associated with this population. Thus, in almost every evaluation, accessory sperm data were characterized by standard deviations approximately twice the value of the mean.

Due to this high variation in accessory sperm numbers, median values were also included in our statistical analysis of accessory sperm data. The high variance in accessory sperm numbers makes statistical determination of changes in the shape of the distribution difficult. The median value, however, which represents the point where 50% of the embryos/ova lie on either side, may be more useful, in a population such as this, to detect shifts in the distribution without changes in shape.

Figure 11 shows the distribution, by accessory sperm number, of all embryos (UFO excluded) recovered during this study (Figure 11 on page 66). Although the shape of the curve did adopt a more normal type distribution, there was still a relatively high proportion of embryos with zero accessory sperm and the mode value only shifted to the one to three range of accessory sperm numbers. Thus, the mean number accessory sperm increased from 10.5 ± 23.8 to 14.4 ± 26.9 and the median value shifted from two to four for the accessory sperm distributions with and without UFO, respectively.

The distribution of embryos/ova by accessory sperm number observed in this study is nearly identical to that presented by Saacke and coworkers (1988b). In their data set approximately 60% of the embryos/ova had 10 or less accessory sperm and several embryos had greater than 100 sperm.

The factors regulating or controlling accessory sperm numbers and resulting in these extremely large distributions are unknown. With results of this study and that of other researchers suggesting that accessory sperm number are correlated with fertilization status/embryo quality, determination of these controlling variables could be important to establishing appropriate sperm numbers per artificial insemination dose (Saacke et al., 1988b) and/or increasing reproductive efficiency.

Embryo Recovery

The overall embryo/ovum recovery rate for this series of experiments was 70% with 192 embryos/ova recovered from 273 inseminations. The overall fertilization rate was 73% with 135 of 186 embryos/ova showing signs of cleavage and fertilization. Six (3%) of the 192 embryos/ova recovered during this study were recovered as ruptured zonae pellucidae. The proportion or ruptured zonae recovered in several other studies has been about 5% (Kidder et al., 1954; Bearden et al., 1956; Roche et al., 1981). Hawk and Tanabe (1986) observed a much higher percentage with 17 of 85 ova (20%) recovered as ruptured zonae pellucidae. The lower percentage of ruptured zonae pellucidae recovered during this study is likely due to the less tramatic nonsurgical flushing techniques utilized in these experiments. Hawk and Tanabe slaughtered cows three to five d after breeding, removed the genital tract and flushed the oviducts and uterus with saline solution. This procedure required much more physical manipulation of the reproductive tract and thus may result in an increased incidence of damaged embryos/ova.

Conclusions

Retrograde sperm blockage at the time of insemination did not improve accessory sperm numbers or fertilization rates. Although accessory sperm numbers were not increased by blocking, it cannot be concluded that blocking had no effects on sperm retention and/or sperm transport. It is possible that blocking did increase sperm retention but the indirect measure of sperm transport, accessory sperm, was not sensitive enough to detect these changes. It is apparent, however, from the results of this study that blocking retrograde flow had no adverse effects on accessory sperm numbers or fertilization rates.

Blocking at the time of insemination can apparently have an adverse effect on embryo quality as shown in Experiment 1. In this experiment, a higher proportion of degenerate embryos were obtained from blocked inseminations when compared with embryos recovered from control inseminations. This observation may be due to adverse effects of the blocking apparatus on the uterine/oviductal environment which could ultimately result in an impairment of their ability to sustain embryonic development. Future attempts to increase sperm retention by blocking retrograde flow should consider these results when designing a blocking apparatus and/or designating the period of the blocking interval.

Sperm dose had no significant effect on accessory sperm numbers or fertilization status/embryo quality in this study.

Semen quality showed a trend towards increasing fertilization status/embryo quality; however, the increase was not significant. Semen quality also had no significant effect on accessory sperm number within individual experiments, but, an overall comparison across experiments suggested that accessory sperm numbers may be related to semen quality.

The relationship between embryo quality and accessory sperm numbers appeared vary depending on the quality of semen used to inseminate. Embryos recovered from cows bred with average quality semen had significantly greater responses in quality to increased accessory sperm number than embryos recovered from cows bred with below average quality semen. Thus, it appears that utilization of semen with a low proportion of morphologically abnormal cells may increase fertility by either increasing the proportion of oocytes which ultimately become fertilized and/or by increasing embryo quality and survival rates once fertilization has occurred.

Spermatozoa with severe morphological abnormalities appear to be selected against by the sperm transport mechanisms of the female and/or the zona pellucida of the ovum. Based on accessory sperm, the degree or intensity of this selection process is dependant upon the severity of the abnormality in question. Spermatozoa with slight or subtle head abnormalities of all types are frequently observed as accessory sperm in the zona pellucida of embryos/ova while severely abnormal cells with misshapen heads are completely excluded.

Accessory sperm numbers appear to be correlated with fertilization status/embryo quality. Unfertilized ova consistantly have mean and median values of zero accessory sperm. Viable quality embryos (excellent-good & fair-poor) consistantly have the highest mean and median number accessory sperm while degenerates appear to be intermediate in number. These observations suggest that fertilization rates and/or embryo survival can be improved by increasing accessory sperm numbers per embryo/ovum.

Frequency evaluation of embryos/ova by accessory sperm numbers reveals a population with a highly skewed distribution. While approximately 80% of embryos/ova have 15 or less accessory sperm, many are scattered through an extremely long tail that extends well over 100 accessory sperm per embryo. This type of distribution results in means within the lower distribution of accessory sperm numbers and extremely high variances that yield a standard deviation twofold to threefold greater than the mean. Thus, detecting significant differences between treatments within populations such as this requires special statistical treatment of the data to stablize these variances and/or extremely large sample sizes. In this study, the variances were stabilized by transforming accessory sperm data to Log_{10} (Value + 1). Due to the high variance associated with this type of distribution, median values may be more indicative of accessory sperm populations than the means and standard deviations.

Evidence from this and other studies suggests that accessory sperm numbers are related to fertilization rates, embryo quality, and embryo survival. Future research regarding accessory sperm should be designed to determine the factors controlling or regulating accessory sperm numbers per embryo/ovum. Can we override these controlling factors (with fresh and/or high dose of semen) and consistantly produce embryos with high accessory sperm numbers? If so, does the trend towards increased embryo quality continue at these higher accessory sperm values? Further investigation of the relationship between fertilization status/embryo quality may present a more complete understanding of sperm transport, the fertilization process and/or embryonic development, which could ultimately result in the development of new methods or techniques of insemination designed to increase accessory sperm numbers and/or reproductive efficiency in the artificially inseminated bovine.

Bibliography

Allison, A. J. 1972. A comparison of the transport of spermatozoa in spayed and entire ewes. J. Reprod. Fert. 31:415-423.

Austin, C. R. 1956. Cortical granules in hamster eggs. Exp. Cell Res. 10:533-540.

Austin, C. R. and A. W. H. Braden. 1956. Early reactions of rodent egg to spermatozoan penetration. J. Exp. Biol. 33:358-365.

Ballachey, B. E., W. D. Hohenboken and D. P. Evenson. 1987. Heterogeneity of sperm nuclear chromatin structure and its relationship to fertility. Biol. Reprod. 36:915-925.

Barros, C. and R. Yanagimachi. 1971. Induction of zona reaction in golden hamster eggs by cortical granule material. Nature 233:268-269.

Barros, C. and R. Yanagimachi. 1972. Polyspermy preventing mechanisms in the golden hamster egg. J. Exp. Zool. 180:251-266.

Bearden, H. J., W. M. Hansel and R. W. Bratton. 1956. Fertilization and embryonic mortality rates of bulls with histories of either low or high fertility in artificial breeding. J. Dairy Sci. 39:312-318.

Bishop, M. W. H., R. C. Campbell, J. L. Hancock and A. Walton. 1954. Semen characteristics and fertility in the bull. J. Agric. Sci., Camb. 44:227-248.

Chang, M. C. and D. M. Hunt. 1956. Effects of proteolytic enzymes on the zona pellucida of fertilized and unfertilized mammalian eggs. Exp. Cell Res. 11:497-499.

Conley, H. H. and H. W. Hawk. 1970. Intensification by intrauterine devices of sperm loss from the sheep uterus. Biol. Reprod. 2:401-407.

De Felici, M. and G. Siracusa. 1982. Spontaneous hardening of the zona pellucida of mouse oocytes during in vitro culture. Gamete Res. 6:107-113.

Dobrowolski, W. and E. S. E. Hafez. 1970. Transport and distribution of spermatozoa in the reproductive tract of the cow. J. Anim. Sci. 31:940-943.

Döcke, F. 1961. Über den einfluss des vegetativen nervensystems auf die uterusmotilität beim rind. Proc. IV Int. Congr. Anim. Reprod. Art. Insem. (The Hague) II:293.

Dresdner, R. D. and D. F. Katz. 1981. Relationship of mammalian sperm motility and morphology to hydrodynamic aspects of cell function. Biol. Reprod. 25:920-930.

El-Banna, A. A. and E. S. E. Hafez. 1970. Sperm transport and distribution in rabbit and cattle female tract. Fert. Ster. 21:534-540.

Evenson, D. P., Z. Darzynkiewicz and M. R. Melamed. 1980. Relation of mammalian sperm chromatin heterogeneity to fertility. Nature 210:1131-1133.

Eyestone, W. H. and N. L. First. 1989. Variation in bovine embryo development in vitro due to bulls. Therio. 31:191.

First, N. L., R. E. Short, J. B. Peters and F. W. Stratman. 1968. Transport and loss of boar spermatozoa in the reproductive tract of the sow. J. Anim. Sci. 27:1037-1040.

Fol, H. 1877. Sur le premier développement d'une Étoile de mer. Compt. Rend. Acad. Sci., Paris. 84:357-360.

Gallagher, G. R. and P. L. Senger. 1989. Concentrations of spermatozoa in the vagina of heifers after deposition of semen in the uterine horns, uterine body or cervix. J. Reprod. Fert. 86:19-25.

Gianfortoni, J. F. and B. J. Gulyas. 1985. The effects of short-term incubation (aging) of mouse oocytes on in vitro fertilization, zona solubility, and embryonic developement. Gamete Res. 11:59-68.

Gwatkin, R. B. L., D. T. Williams, J. F. Hartman and M. Kniazuk. 1973. The zona reaction of hamster and mouse eggs: production in vitro by a trypsin-like protease from cortical granules. J. Reprod. Fertil. 32:259-267.

Gwatkin, R. B. L., G. H. Rasmusson and D. T. Williams. 1976. Induction of the cortical reaction in hamster eggs by membrane-active agents. J. Reprod. Fert. 47:299-303.

Harper, M. J. K. 1973. Relationship between sperm transport and penetration of eggs in the rabbit oviduct. Biol. Reprod. 8:441-450.

Hawk, H. W. 1970. Rapid disruption of sperm transport mechanisms by intra-uterine devices in the ewe. J. Reprod. Fert. 23:139-142.

Hawk, H. W. 1975. Hormonal control of changes in the direction of uterine contractions in the estrous ewe. Biol. Reprod. 12:423-430.

Hawk, H. W. 1987. Transport and fate of spermatozoa after insemination of cattle. J. Dairy Sci. 70:1487.

Hawk, H. W. and H. H. Conley. 1971. Loss of spermatozoa from the reproductive tract of the ewe and intensification of sperm 'breakage' by progestagen. J. Reprod. Fert. 27:339-347.

Hawk, H. W. and H. H. Conley. 1985. Effect of prostaglandin $F_{2\alpha}$, phenylephrine and ergonovine on uterine contractions in the ewe. J. Anim. Sci. 60:537-543.

Hawk, H. W., H. H. Conley, R. J. Wall and R. O. Whitaker. 1988. Fertilization rates in superovulating cows after deposition of semen on the infundibulum, near the uterotubal junction or after insemination with high numbers of sperm. Therio. 29:1131

Hawk, H. W. and B. S. Cooper. 1984. Improvement by ergonovine of sperm transport, fertilization and pregnancy rates in ewes in natural or prostaglandin-induced estrus. J. Anim. Sci. 59:754-763.

Hawk, H. W. and T. Y. Tanabe. 1986. Effect of unilateral cornual insemination upon fertilization rate in superovulating and single-ovulating cattle. J. Anim. Sci. 63:551

Hays, R. L. and N. L. VanDemark. 1951. Stimulatory action of breeding on the release of oxytocin as measured by intramammary pressure. J. Dairy Sci. 34:496.

Hays, R. L. and N. L. VanDemark. 1953. Effects of oxytocin and epinephrine on uterine motility in the bovine. Am. J. Physiol. 172:557-560.

Heydon, R. A. and N. R. Adams. 1979. Comparative morphology and mucus histochemistry of the ruminant cervix: Differences between crypt and surface epithelium. Biol. Reprod. 21:557-562.

Hillery, F. L., J. J. Parrish and N. L. First. 1990. Bull specific effect on fertilization and embryo development in vitro. Therio. 33:249.

Howe, G. R. and D. L. Black. 1963. Spermatozoan transport and leucocytic responses in the reproductive tract of calves. J. Reprod. Fert. 6:305-311.

Hunter, R. H. F. 1984. Pre-ovulatory arrest and peri-ovulatory redistribution of competent spermatozoa in the isthmus of the pig oviduct. 72:203-211.

Hunter, R. H. F. and R. Nichol. 1983. Transport of spermatozoa in the sheep oviduct: Preovulatory sequestering of cells in the caudal isthmus. J. Exp. Zool. 228:121-128.

Hunter, R. H. F. and I. Wilmut. 1984. Sperm transport in the cow: peri-ovulatory redistribution of viable cells within the oviduct. Reprod. Nutr. Dev. 24:597.

Karabinus, D. S. and R. G. Saacke. 1987. Selection of spermatozoa across the bovine cervix. J. Dairy Sci. 70(Supp. 1):248.

Kidder, H. E., W. G. Black, J. N. Wiltbank, L. C. Ulberg and L. E. Casida. 1954. Fertilization rates and embryonic death rates in cows bred to bulls of different levels of fertility. J. Dairy Sci. 37:691.

Krzanowska, H. 1974. The passage of abnormal spermatozoa through the uterotubal junction of the mouse. J. Reprod. Fert. 38:81-90.

Krzanowska, H. and E. Lorenc. 1983. Influence of egg investments on in-vitro penetration of mouse eggs by misshapen spermatozoa. J. Reprod. Fert. 68:57-62.

Larsson, B. 1986. Transperitoneal migration of spermatozoa in heifers. J. Vet. Med. A. 33:714-718.

Larsson, B. and K. Larsson. 1985. Distribution of spermatozoa in the genital tract of artificially inseminated heifers. Acta Vet. Scand. 26:385-395.

Lightfoot, R. J. and B. J. Restall. 1971. Effects of site of insemination, sperm motility and genital tract contractions of transport of spermatozoa in the ewe. J. Reprod. Fert. 26:1-13.

Lindner, G. M. and R. W. Wright. Jr. 1983. Bovine embryo morphology and evaluation. Theriogenology. 20:407-416.

Linford, E., F. A. Glover, C. Bishop and D. L. Stewart. 1976. The relationship between semen evaluation methods and fertility in the bull. J. Reprod. Fert. 47:283-291.

Longo, F. J. 1981. Changes in the zonae pellucidae and plasmalemma of aging mouse eggs. Biol. Reprod. 25:399-411.

Mahadevan, M. M., A. O. Trounson, and J. F. Leeton. 1983. The relationship of tubal blockage, infertility of unknown cause, suspected male infertility, and endometriosis to success of in vitro fertilization and embryo transfer. Fert. Ster. 40:755-762.

Matthews, M. K., Jr. and N. T. Adler. 1978. Systematic interrelationship of mating, vaginal plug position, and sperm transport in the rat. Physiol. Behav. 20:303-309.

Mattner, P. E. 1963. Spermatozoa in the genitlal tract of the ewe. II. Distribution after coitus. Aust. J. Biol. Sci. 16:688-694.

Mattner, P. E. 1968. The distribution of spermatozoa and leucocytes in the female genital tract in goats and cattle. J. Reprod. Fert. 17:253-261.

Mattner, P. E. 1969. Phagocytosis of spermatozoa by leucocytes in bovine cervical mucus in vitro. J. Reprod. Fert. 20:133-134.

Mattner, P. E. 1973. The cervix and its secretions in relation to fertility in ruminants. Pages 339-350 in The biology of the cervix. R. J. Blandau and K. S. Moghissi, ed. Univ. Chicago Press.

Mattner, P. E. and A. W. H. Braden. 1963. Spermatozoa in the genital tract of the ewe. I. Rapidity of transport. Aust. J. Biol. Sci. 16:473-481.

Miller, D. M., F. Hrudka, W. F. Cates and R. J. Mapletoft. 1982. Infertility in a bull with a nuclear sperm defect: A case report. Therio. 17:611-621.

Mitchell, J. R., P. L. Senger, and J. L. Rosenberger. 1985. Distribution and retention of spermatozoa with acrosomal and nuclear abnormalities in the cow genital tract. J. Anim. Sci. 61:956.

Mortimer, D. 1977. The survival and transport to the site of fertilization of diploid rabbit spermatozoa. J. Reprod. Fert. 51:99-104.

Morton, D. B. and T. D. Glover. 1974. Sperm transport in the female rabbit: The effect of inseminate volume and sperm density. J. Reprod. Fert. 38:139-146.

Moser, F. 1939. Studies on a cortical layer response to stimulating agents in the Arbacia egg. J. Exptl. Zool. 80:423

Mullins, K. J. and R. G. Saacke. 1989. Study of the functional anatomy of bovine cervical mucosa with special reference to mucus secretion and sperm transport. Anat. Rec. 225:106-117.

Nelson, V. E., E. P. Aalseth, C. H. Hawman, G. D. Adams, L. J. Dawson, and R. W. McNew. 1987. Sperm discharge and distribution within the cows reproductive tract after A.I. J. Anim. Sci. 65(Suppl. 1):401, Abstr.

Overstreet, J. W. and G. W. Cooper. 1975. Reduced sperm motility in the isthmus of the rabbit oviduct. Nature (Lond.) 258:718-719.

Overstreet, J. W. and G. W. Cooper. 1978a. Sperm transport in the reproductive tract of the female rabbit: I. The rapid transit phase of transport. Biol. Reprod. 19:101-114.

Overstreet, J. W. and G. W. Cooper. 1978b. Sperm transport in the reproductive tract of the female rabbit: II. The sustained phase of transport. Biol. Reprod. 19:115-132.

Overstreet, J. W. and G. W. Cooper. 1979. Effect of ovulation and sperm motility on the migration of rabbit spermatozoa to the site of fertilization. J. Reprod. Fert. 55:53-59.

Quinlivan, T. D. and T. J. Robinson. 1969. Numbers of spermatozoa in the genital tract after artificial insemination of progestagen-treated ewes. J. Reprod. Fert. 19:73-86.

Roche, J. F., M. P. Boland, and T. A. McGeady. 1981. Reproductive wastage following artificial insemination of heifers. Vet. Rec. 109:401.

Ruckebusch, Y. and F. Bayard. 1975. Motility of the oviduct and uterus of the cow during the oestrus cycle. J. Reprod. Fert. 43:23-32.

Saacke, R. G., H. Bader, and K. F. Weitze. 1986. Eine Technik zur Ruckgewinnung und Untersuchung von Spermien aus der Zona Pellucida des Rindes. Zuchthyg. 21:148.

Saacke, R. G., J. H. Bame, D. S. Karabinus, K. J. Mullins, and S. Whitman. 1988a. Transport of abnormal spermatozoa in the artificially inseminated cow based upon accessory sperm in the zona pellucida. 11th Intl. Cong. Anim. Reprod. and Artif. Insem. Dublin. 3:292-294.

Saacke, R. G. and C. E. Marshall. 1968. Observations of the acrosomal cap of fixed and unfixed bovine spermatozoa. J. Reprod. Fert. 16:511-514.

Saacke, R. G., R. L. Nebel, D. S. Karabinus, J. H. Bame and K. J. Mullins. 1988b. Sperm transport and accessory sperm evaluation. Proc. 12th Tech. Conf. Artif. Insem. and Reprod. National Association of Animal Breeders. pp. 7-14.

Saacke, R. G. and J. M. White. 1972. Semen quality tests and their relation to fertility. Proc. 4th Tech. Conf. Artif. Insem. Reprod. National Association of Animal Breeders. pp 22-27.

SAS, 1985. SAS User's Guide. Cary, NC: SAS Institute Inc.

Sathananthan, A. H. and A. O. Trounson. 1982. Ultrastructure of cortical granule release and zona interaction in monospermic and polyspermic human ova fertilized in vitro. Gamete Res. 6:225-234.

Segerson, E. C. and D. W. Libby. 1981. Ova fertilization and sperm number per fertilized ovum for selenium and vitamin E-treated Charolais cattle. Therio. 17:333-341.

Settlage, D. S. F., M. Motoshima, and D. R. Tredway. 1973. Sperm transport from the external cervical os to the Fallopian tubes in women: a time and quantitation study. Fertil. Steril. 24:655-661.

Shi, D. S., K. H. Lu and I. Gordon. 1990. Effect of bulls on fertilization of bovine oocytes and their subsequent development in vitro. Therio. 33:324.

Suarez, S. S. 1987. Sperm transport and motility in the mouse oviduct: Observations in situ. Biol. Reprod. 36:203-210.

Suga, T. and S. Higaki. 1971. Studies on uterine secretions in the cow. II. Distribution of spermatozoa and seminal plasma after intra-uterine inseminations in the reproductive tract of the cow during oestrus. Bull. Natl. Inst. Anim. Ind. (Jpn) 24:41.

Sullivan, J. J. and F. I. Elliott. 1968. Bull fertility as affected by an interaction between motile spermatozoa concentration and fertility level in artificial insemination. VI Congr. Int. Reprod. Anim. Insem. Artif. (Paris) II:1307-1310.

Szollosi, D. 1967. Developement of cortical granules and the cortical reaction in rat and hamster eggs. Anat. Rec. 159:431-446.

Tesarik, J., L. Pilka and P. Travnik. 1988. Zona pellucida resistance to sperm penetration before the completion of human oocyte maturation. J. Reprod. Fert. 83:487-495.

Tilbrook, A. J. and D. T. Pearce. 1986. Pattern of loss of spermatozoa from the vagina of the ewe. Aust. J. Biol. Sci. 39:295-303.

Trounson, A. O. and J. Webb. 1984. Fertilization of human oocytes following reinsemination in vitro. Fert. Ster. 41:816-819.

Trounson, A. O., L. R. Mohr, C. Wood and J. F. Leeton. 1982. Effects of delayed insemination on in vitro fertilization, culture and transfer of human embryos. J. Reprod. Fert. 64:285-294.

VanDemark, N. L. and R. L. Hays. 1951. The effect of oxytocin, adrenalin, breeding techniques and milking on uterine motility in the cow. J. Anim. Sci. 10:1083.

VanDemark, N. L. and R. L. Hays. 1952. Uterine motility responses to mating. Am. J. Physiol. 170:518-521.

VanDemark, N. L. and A. N. Moeller. 1951. Speed of spermatozoan transport in reproductive tract of estrous cow. Am. J. Physiol. 165:674-679.

Wabik-Sliz, B. 1979. Number of corticle granules in mouse oocytes from inbred strains differing in efficiency of fertilization. Biol. Reprod. 21:89-97.

Weitze, K. F., D. Rath, C. E. Pena A., C. J. A. Moura and I. Scheid. 1988. Influence of different sperm number and seminal plasma in the inseminate upon number of accessory spermatozoa in pig embryos. Proc. JPVS Cong. Rio de Janero. Nr. 1.

Wilmut, I. and R. H. F. Hunter. 1984. Sperm transport into the oviducts of heifers mated early in oestrus. Reprod. Nutr. Develop. 24:461-468.

Wolf, D. P. 1981. The mammalian egg's block to polyspermy. In Fertilization and Embryonic Developement In Vitro, pp. 183-197. Eds L. Mastroianni & J. D. Biggers. Raven Press, New York.

Wolf, D. P. and M. Hamada. 1976. Age-dependent losses in the penetrability of mouse eggs. J. Reprod. Fert. 48:213-214.

Wolf, D. P. and M. Hamada. 1977. Induction of zonal and egg plasma membrane blocks to sperm penetration in mouse eggs with corticle granule exudate. Biol. Reprod. 17:350-354.

Zenzes, M. T., L. Belkien, J. Bordt, I. Kan, H. P. G. Schneider and E. Nieschlag. 1985. Cytologic investigation of human in vitro fertilization failures. Fert. Ster. 43:883-891.

Appendix A. Composition of Antibiotic-Antimycotic used in flushing procedures

(Gibco Laboratories, Grand Island, N.Y., Cat. No. 600-5245AE).

Prepared with:

10,000 units/ml penicillin G sodium

10,000 mcg/ml streptomycin sulfate

25 mcg/ml amphotericin B as Fungizone

Appendix B. Embryo Quality Evaluation Procedures

Lindner & Wright (1983)

Excellent - an ideal embryo, spherical, symmetrical with cells of uniform size, color and texture.

Good - trivial imperfections such as a few extruded blastomeres, irregular shape, few vesicles.

Fair - definite but not severe problems, presence of extruded blastomeres, vesiculation, few degenerate cells.

Poor - severe problems, numerous extruded blastomeres, degenerate cells, cells of varying sizes, large numerous vesicles but a viable-appearing embryo mass.

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Publications

DeJarnette, J. M., R. G. Saacke, J. Bame, and C. J. Vogler. 1990. Effect of sperm dose and retrograde sperm blockage on accessory sperm number and embryo quality in artificially inseminated cows. J. Anim. Sci. (Abstr., submitted for publication Feb. 1990).

Vogler, C. J., R. G. Saacke, J. H. Bame, and J. M. DeJarnatte. 1990. Effects of testicular thermal insult on viability characteristics of cryopreserved semen. J. Dairy Sci. 73 (Suppl. 1):182.

Vogler, C. J., R. G. Saacke, J. H. Bame, and J. M. DeJarnette. 1990. Effects of elevated testicular temperature on morphological characteristics of ejaculated sperm. J. Anim. Sci. (Abstr., submitted for publication Feb. 1990).

Professional Societies

Society of Sigma Xi

American Dairy Science Association

American Society of Animal Science

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