The Effects of the Cervix on the Transport of Morphologically Abnormal Spermatozoa in the Female Bovine

by

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(ABSTRACT)

Two studies were conducted to investigate the role of the bovine cervix in filtering abnormal sperm. In Study 1, semen containing high levels of abnormal sperm was vaginally deposited in 12 cows 80 hours after prostaglandin $F_2\alpha$ treatment. At slaughter, 4-, 8- or 12 hours post-insemination, sperm were flushed from the excised uteri with fixative. Pooled across times post-insemination, viability was greater for uterine vs inseminate sperm, based upon vital smears prepared from inseminate and uterine flush samples. Uterine levels of normal sperm, determined by differential interference contrast (DIC) microscopy, were greater than were inseminated. In Study 2, heifers were estrus synchronized in pairs using prostaglandin $F_2\alpha$, then inseminated with semen containing high levels of abnormal sperm. In each pair, semen of high viability (Experiment 1, $n=10$) or low viability (Experiment 2, $n=6$) was deposited vaginally in one heifer and within the uterine corpus of the other. Using DIC microscopy, viability and morphology were coincidentally determined for sperm in samples from the fixed inseminate and the retrograde mucus, vaginal mucus, cervix, uterus and *in vitro* incubation of inseminate recovered and fixed 12 hours post-insemination. Uterine sperm quality did not differ between insemination sites, except lower uterine levels of live abnormal sperm after intrauterine vs vaginal insemination of low viability semen probably due to disproportionately low viability of one abnormality. Sperm viability was enhanced and morphology unchanged in the uterus vs low viability inseminate, while sperm viability was unchanged and abnormal sperm subtly reduced in uterus vs high viability inseminate. Greater levels of live and live normal sperm were found deeper between cervical folds than at apical aspects of folds. Vaginal mucus sperm viability was lower compared to other tract locations and inseminate,

especially after high viability insemination. Compared to inseminate viability, retrograde mucus sperm viability was high after vaginal insemination and low after intrauterine insemination. Differential death of abnormal vs normal sperm neither with incubation of inseminate *in vitro* nor, presumably, *in vivo*. Results show little evidence of cervical filtration based upon sperm morphology. Sperm retention in the female tract was predominately related to sperm viability with only very subtle morphology effects.

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Introduction

In mammals, the biological function of mating is to deposit semen in the female reproductive tract such that union of sperm and oocyte(s) can occur to yield viable offspring. In the time between deposition of the semen and the fertilization of the oocyte(s), a series of events in the female genital tract affect the transport of sperm from the site of semen deposition to the site of fertilization. Sperm transport in the female consists of spatial, temporal, quantitative and qualitative aspects that interact, presumably, to increase the probability of viable fertilization. An understanding of the aspects of sperm transport should yield insights to methods for increasing the chances of fertilization and the identification of gamete incompetence. In this context, fertility is determined by the measures that gauge the potential for success of sperm-oocyte encounters or by the ability of the respective gametes to complete the task should such an encounter occur. Gametes possessing impaired functional competence in reaching the site of fertilization, completing fertilization and/or sustaining the early embryo would be expected to adversely affect fertility.

Two distinct anatomical locations are recognized as the sites of semen deposition in the bovine: the anterior vagina against the external cervical os in natural mating, and the uterine corpus in artificial insemination. The cervix presents a naturally-imposed barrier to the passage of vaginally deposited sperm that is bypassed in artificial insemination. In the

past three decades evidence has been presented which indicates the cervix functions to provide i) a reservoir from which sperm migrate to effect fertilization, ii) a haven for sperm from phagocytosis and iii) a possible means for the removal of incompetent sperm. Circumvention of the cervix in artificial insemination may eliminate a natural mechanism employed by the female to enhance the opportunity for successful fertilization by protecting desirable sperm and excluding undesirable sperm. Although circumstantial evidence suggests cervical involvement in the removal of abnormal sperm from the female tract, it has not been shown that the cervix is directly responsible for creating a difference between the proportions of abnormal sperm in the inseminate and in the uterus after vaginal semen deposition and subsequent cervical passage.

The purpose of this study was to investigate the potential role of the bovine cervix in the transport of vaginally deposited, morphologically abnormal sperm by pursuing the following specific objectives:

a. Determine whether the proportion and type of morphologically abnormal sperm in the uterus was different from that vaginally inseminated,
b. Differentiate uterine from cervical effects on the proportion and type of morphologically abnormal sperm in the uterus after vaginal insemination,
c. Evaluate the relationship between the viability of morphologically abnormal sperm and their transport and retention, and
d. Determine how the proportion of live, morphologically abnormal sperm differs among quarters of the cervix following vaginal semen deposition.

Review of Literature

Historical

The study of sperm transport can easily be regarded as a discipline of the 20th century; one whose evolution has been driven by the need to answer questions generated by the adoption of artificial breeding techniques, the search for artificial birth control methods and by the development of laboratory optics and instrumentation of sufficient sensitivity to reliably address those questions. The origins of the study of sperm transport in mammals can be traced to a report on the independent movement of spermatozoa and later observations on sperm ascent in the reproductive tract of the female rabbit by Antony van Leeuwenhoeck (1677; 1685; cited in Blandau, 1969). That author proposed that the observations of van Leeuwenhoeck formed the basis for the theory that sperm traversed the uterus by their own motile abilities. Studies conducted during the mid 1800s in dogs and guinea pigs (Hausmann, 1840; Leuckart, 1853; cited in Blandau, 1969), however, appeared to minimize the importance of sperm motility for their passage from the vagina towards the oviducts. Bischoff (1845; cited in Evans, 1933) found sperm throughout the uterus but not in the oviducts of a bitch sacrificed immediately after mating, which indicated some mechanism, at least working in conjunction

with sperm motility, had effected the rapid movement of sperm from the site of deposition toward the oviducts.

Despite those early reports, Evans (1933) stated that sperm motility alone was extensively held to be responsible for the ingress of sperm through the female reproductive tract until the observations in the rat suggested a role was played by genital tract contractions (Hartman and Ball, 1930). Using a fistulated uterus technique, Florey and Walton (1932) confirmed the observations of Hartman and Ball (1930) showing the immediate appearance of semen in the uterus after copulation in the rat and in the guinea pig. In the rabbit, however, Parker (1930) concluded that sperm were transported to the uterus by vaginal contractions and traversed the uterus under their own power. Florey and Walton's (1932) work in the rabbit lent support to Parker's (1930) conclusion from the standpoint of the time required for sperm travel. Evans (1933) demonstrated the rapid ingress of vaginal sperm to the uterus using a fistulated uterus technique in the dog.

Small animals, such as the dog, were used to study sperm transport due to availability and low cost, but the introduction of artificial insemination methods in the livestock species necessitated study of the process in the specific species in question (Evans, 1933). Researchers were cautioned by Evans (1933) against making generalizations concerning sperm transport across species because of the negative effect those generalizations might have on the results of breeding practices (i.e. artificial insemination, Iwanow, 1930) that had been just recently introduced at that time.

To effectively use artificial insemination or hand mating in the breeding of livestock, it became apparent that understanding the processes involved in sperm passage to the oocyte was necessary to obtain best fertility results. Although the time of ovulation relative to estrus and the optimum time of mating for best fertility had been adequately defined by the early 1940s (Woodman and Hammond, 1925; Andreev, 1940; Brewster et al., 1940; Brewster and Cole, 1941; Nalbandov and Casida, 1942), it was still unclear as to what happened to sperm after semen was deposited in the female. While the various regions of the bovine tract appeared to have differing effects on the sperm they contained (Woodman and Hammond, 1925;

Kirillov, 1937; Beshlebnov, 1938; Sergin et al., 1940), little was known about the rate of sperm travel through the cow's reproductive tract and differences of opinion existed on the involvement of uterine contractions vs sperm motility in sperm transport (Beshlebnov, 1938; Brewster et al., 1940; Sergin et al., 1940). Conflicting reports on the transport of dead sperm (Beshlebnov, 1938; Brewster et al., 1940) were early indications that sperm quality may have some effect on sperm transport. The clinical observations of Williams and Savage (1925, 1927), Lagerlöff (1934, 1936), Addis (1937), Generales (1938) and Sciuchetti (1938) indicated that abnormally shaped sperm negatively affected fertility, but a significant statistical relationship between sperm abnormalities and fertility could not be shown (Swanson and Herman, 1941; Laing, 1945a). At that time, no information was available on the transport of morphologically abnormal spermatozoa in the bovine female.

In light of what is known today, the components of sperm transport were poorly understood in the 1940s. An examination and understanding of the process in the female that move sperm from the site of deposition to the site of fertilization would elucidate elements affecting fertility.

Because the bovine is an economically important species, information pertinent to the improvement of reproductive efficiency is desirable. The present study addresses the effect of the bovine cervix on the transport of morphologically abnormal sperm due to the apparent negative relationship of abnormal sperm with fertility and because the cervix is bypassed in artificial insemination of this species. These factors necessitate, therefore, a discussion of cervical structure and function, the transport of sperm in the female, morphological aspects of semen quality and their fertility effects, and the transport of abnormal sperm. This review of literature will consider those topics as they relate to the bovine and include relevant information from other species.

Cervix and Cervical Mucus

Anatomically, the cervix is the most caudal segment of the uterus and arises embryonically from the paramesonephric ducts with the uterus and anterior vagina (Hafez, 1987). It is, however, a morphologically and physiologically distinct organ (Roark and Herman, 1950) whose function in the transport of sperm, in those species where vaginal semen deposition naturally occurs, is dependant on that morphology and physiology.

In the bovine, the cervix is roughly cylindrical, approximately 10 cm long (Parkes, 1960) with a wall thickness of about 3cm (Parkes, 1960; Sisson, 1975). The cervical canal is usually characterized by four somewhat spirally arranged, but incompletely annular folds, the most caudal of which projects into the vagina as the "portio" with the external cervical os at its center (Parkes, 1960). The cervical mucosa is thrown into large longitudinal folds (Woodman and Hammond, 1925; Herrick, 1951; Mullins and Saacke, 1982, 1989) which are taller, more numerous and less regular in size in the cranial portion of the cervix, (Parkes, 1960; Heydon and Adams, 1979) and smaller, secondary folds, (Herrick, 1951; Heydon and Adams, 1979; Mullins and Saacke, 1982, 1989) both of which can extend the length of the organ (Woodman and Hammond, 1925; Mullins and Saacke, 1982, 1989). Superimposed on the larger folds are tertiary folds (Herrick, 1951) or grooves (Mullins and Saacke, 1989) and often quaternary folds (Herrick, 1951), arranged longitudinally over the entire surface of the mucosa (Herrick, 1951; Mullins and Saacke, 1989). The bovine cervical mucosa has been variously described as arranged into folds (Herrick, 1951; Lightfoot and Restall, 1971; Sisson, 1975; Heydon and Adams, 1979; Mullins and Saacke, 1982, 1989) or villi (Mattner, 1966; Mattner and Braden, 1969a) and containing crypts (Mattner, 1966; Herrick, 1951; Mullins and Saacke, 1982) or crypts of folds (Wordinger et al., 1972). Mucosal folding (plica; Roark and Herman, 1950) gives the cervix a distinct fern-like appearance (plica palmata; Parkes, 1960) when viewed in cross section. The presence of simple saccular (Hammond, 1927; Roark and Herman, 1950; Mattner, 1966) or branching tubular (Cole, 1930; Roark and Herman, 1950; Mattner, 1966) cervical glands in the

bovine has been reported; however, reports to the contrary also exist (Herrick, 1951; Sisson, 1975; Heydon and Adams, 1979; Mullins and Saacke, 1989).

The cervical mucosa is composed of epithelium and lamina propria underlaid by a muscularis composed chiefly of dense connective tissue with smooth muscle dispersed in irregular bundles (Roark and Herman, 1950; Leeson and Leeson, 1980). The epithelium is composed of nonciliated simple columnar cells which produce the cervical secretion interspersed with ciliated columnar cells (Wordinger et al., 1972).

The character of cervical changes that occur with the stage of the estrous cycle in the bovine have been described by several authors (e.g. Woodman and Hammond, 1925; Cole, 1930; Roark and Herman, 1950; Herrick, 1951; Heydon and Adams, 1979; Mullins and Saacke, 1989), a synthesis of which is presented here. During the luteal phase, height of the columnar epithelium is generally reduced showing limited secretory activity (Woodman and Hammond, 1925; Cole, 1930; Roark and Herman, 1950; Herrick, 1951). Secretion occurs from the apices and basal regions of the secondary indentations (Herrick, 1951; Heydon and Adams, 1979) or grooves (Mullins and Saacke, 1989) on the larger folds, producing a relatively small amount of thick, viscid mucus (Woodman and Hammond, 1925; Roark and Herman, 1950; Herrick, 1951) composed primarily of neutral- (Mullins and Saacke, 1989) and sulfomucins (Heydon and Adams, 1979; Mullins and Saacke, 1989). The lamina propria and muscularis appear dense and uncongested (Cole, 1930; Herrick, 1951) and the cervix exhibits maximum tone (Woodman and Hammond, 1925; Roark and Herman, 1950) and a constricted lumen at this time (Woodman and Hammond, 1925). With the approach of estrus, hyperemia progressively ensues (Woodman and Hammond, 1925; Cole, 1930; Herrick, 1951) resulting in a loosening and increasingly edematous lamina propria (Woodman and Hammond, 1927; Roark and Herman, 1950; Herrick, 1951) and muscularis (Roark and Herman, 1950), cervical relaxation (Woodman and Hammond, 1925; Roark and Herman, 1950) and dilation of the cervical canal (Woodman and Hammond, 1925). The columnar epithelium becomes more uniformly tall and active (Woodman and Hammond, 1925; Cole, 1930; Roark and Herman, 1950; Herrick, 1951), secreting a progressively more fluid mucus in increasing quantities (Woodman and Hammond, 1925;

Roark and Herman, 1950; Herrick, 1951). The decreased viscosity results from the secretion of a greater proportion of sialomucin than neutral- (Mullins and Saacke, 1989) or sulfomucin (Heydon and Adams, 1979; Mullins and Saacke, 1989), being produced primarily from the basal aspects of the secondary indentations (Heydon and Adams, 1979) or grooves (Mullins and Saacke, 1989). Hyperemia reaches its maximum 12-24 hours before the end of estrus (Roark and Herman, 1950), and after ovulation shows a gradual decrease to minimum values by day 7 to 10 of the cycle (Roark and Herman, 1950; Herrick, 1951). Mucus quantity and flow elasticity are greatest early in estrus and progressively decrease with increased time from the onset of estrus, while viscosity does the opposite (Scott-Blair et al., 1941; Roark and Herman, 1950).

That the character of cervical mucus changes with the stage of the estrous cycle has been well established and these changes can be used to indicate the reproductive status in the bovine (Woodman and Hammond, 1925; Scott-Blair, 1941; Grobellar and Kay, 1985). The cyclic nature of bovine cervical mucus has been shown to be under the control of the ovarian steroids (Glover, 1960; Heydon and Adams, 1979) and can act as an agent or a barrier to conception (Linford, 1974). Woodman and Hammond (1925) gave early recognition to the importance of "liquefaction" of mucus in the transport of sperm from the vagina.

Cervical mucus is a hydrogel composed of a glycoprotein matrix that supports water and other soluble material (Linford, 1974), and can be viewed as having a gel and a soluble portion. The gel is a carbohydrate-rich glycoprotein fraction of the mucoid variety comprising 45% of the total protein while the soluble component contains serum proteins and other materials to make up the remaining 55% of the total protein (Gibbons and Mattner, 1971; Hamana et al., 1971). Gibbons (1959a) found cervical mucus glycoprotein to be of the epithelial glycoprotein type having a polypeptide backbone characteristically high in serine, threonine and proline, and having a large number of heterosaccharide side chains. Cervical mucus is referred to as a polydisperse glycoprotein because, although chemically similar, the molecules differ in molecular weight, size and arrangement of the side chains, and can be envisioned as a "spectrum of glycoproteins" with molecular dissimilarity (Gibbons and Mattner,

1971; Syner and Moghissi, 1971). Because of their macromolecular architecture, cervical, gastric, submaxillary and respiratory mucins are described as linear flexible chains (Sheehan and Carlstedt, 1987) whose behavior is similar to that of random coiled polymers (Gibbons, 1959b). The rheologic (flow) properties of mucus are governed by the glycoprotein architecture (Doehr and Moghissi, 1973) and are quantitated by measurements of viscosity, flow elasticity, spinnbarkeit, thixotropy and tack (Moghissi, 1972). The rheologic (flow) properties of mucus vary with the stage of the estrous cycle to yield a low viscosity secretion at estrus (Scott-Blair et al., 1941) that is easily penetrated by sperm (Roark and Herman, 1950; Gaddum-Rosse et al., 1980b).

The current model of mucus glycoprotein structure describes a loose enmeshment between randomly coiled glycoprotein macromolecules having inter- and intra- chain entanglements (Lee et al., 1977; Gaddum-Rosse et al., 1980a, b) as opposed to the previously proposed rigid network made up of aggregates of long glycoprotein molecules (micelles) oriented in a relatively paralleled fashion and stabilized by interchain disulfide bonds involving a separate protein bridging the chains (Gibbons and Mattner, 1966; Odeblad, 1968; Doehr and Moghissi, 1973). The matrix formed by the entanglement of the coiled macromolecules supports a fluid composed of water, dissolved electrolytes, carbohydrates (glycogen, glucose and polysaccharides), lipids, citrate urea, and macromolecules such as enzymes, serum proteins (α, β and δ globulins and albumen) and other proteins and peptides (Schumacher, 1971; Gibbons and Mattner, 1971). During the follicular phase, mucus content of water (Hamana et al., 1971), total protein, (Roychaudhary and Razdan, 1965; Elstein and Pollard, 1968), glucose, (Weed and Carrera, 1970), alkaline phosphatase (Smith, D. C. et al., 1970) and sialic acid (Hamana et al., 1971) increase while cellularity (Austin, 1975), peroxidase (Linford, 1974) and serum protein (Agrawal et al., 1978) decrease. The shift in mucus chemical constituents with approaching estrus is accompanied by a shift in the type of mucin produced, from almost exclusive neutral- and sulfomucin in the luteal phase to those types in addition to sialomucin (Heydon and Adams, 1979; Mullins, 1987) which helps to explain the rheologic properties of estrus mucus. While sialomucin, due to the charge of its sialic acid residues, tends to bind

water molecules (Daunter, 1984), this fact alone does not completely explain the hydration of estrus vs luteal phase mucus. Tam and Verdugo (1981) demonstrated that luteal phase mucus could be hydrated, under physiological pH and NaCl conditions *in vitro*, to exhibit estrus mucus rheologic properties. These authors proposed that mucus hydration may be controlled by a Donnan equilibrium process. They explained that the movement of ions, soluble proteins and water across the cervical mucosa, in conjunction with the proteins and ions within the secreted mucus, could be the physiological controls that determined hydration. The structural proteins of mucus, acting as trapped polyions, would supply the osmotic drive necessary to move water from the edematous cervical mucosa into the matrix of the secreted mucin to hydrate it. Thus it appears that mucus hydration *in vivo* results from the physiological condition of the cervical mucosa, the chemical composition of the secreted mucin and physical laws working in concert to produce the unique change in cervical mucus associated with estrus and the transport of sperm.

The changes in the cervix and its secretion that coincide with estrus and impending ovulation are not chance events but rather, apparently, function to enhance the probability of sperm-oocyte encounters. Estrus mucus is easily penetrated by viable spermatozoa and is one of the first external manifestations of the females' cooperation in the transport of gametes to effect fertilization.

Sperm Transport

For the species in which vaginal semen deposition occurs, the cervix, uterus and uterotubal junction pose physical barriers of varying degrees that sperm must overcome before reaching the site of fertilization (Robinson, 1975). In natural mating, due to the coiling of the glans penis at ejaculation, bull semen is distributed in a circular pattern about the anterior vagina and fornix (Seidel and Foote, 1969). The semen tends to pool in the fornix vagina after

ejaculation and it is from this anatomical location that sperm transport begins (Hafez, 1974; Salisbury et al., 1978).

Sperm transport in the female can be thought of as having four components: rapid sperm transport, sustained sperm transport, formation of sperm reservoirs and loss of sperm from the genital tract. Although the components appear as distinct and separate entities, they in fact overlap or coincide in time. Each component has spatial, temporal, quantitative and qualitative aspects that define it. The spatial and temporal aspects describe changes in physical location of sperm in the female tract and the rate of those changes in location, while the quantitative and qualitative aspects deal with the numbers and characteristics of the sperm being transported.

Rapid sperm transport

The rapid phase of sperm transport in the bovine has been shown to result in the passage of sperm to the oviducts within 2.5 and 11 minutes after intracervical insemination (Van Demark and Moeller, 1950, 1951), and within 13 minutes after vaginal insemination (Howe and Black, 1963) regardless of the motile status of sperm in the inseminate in the former case. Mattner and Braden (1963) found similar results in the ewe where live sperm and inert particulate matter were found in the oviducts 8 to 15 minutes after insemination with some sperm having passed completely out of the oviduct and into the body cavity. Subsequent *in vivo* and *in vitro* work by Van Demark's group suggested that the effect was a result of mating or insemination induction of oxytocin release which stimulated contractions of the uterine musculature (Van Demark and Hays, 1951, 1952, 1954; Hays and Van Demark, 1952, 1953a,b; Moeller and Van Demark, 1955). Stress has been shown to eliminate the rapid phase of sperm transport in the cow (Van Demark and Hays, 1952) and ewe (Mattner, 1963a) probably by the elimination of uterine contractions by the negatory effects of epinephrine on oxytocin action (Einarsson, 1980). The elimination of rapid sperm transport does not affect the long term distribution of sperm in the female reproductive tract since rapid sperm transport has no effect on the number of sperm that are later found to populate the cervix and only a transient effect

on sperm numbers in the cervix and oviducts (Mattner and Braden, 1969a). Thus, the role of rapid sperm transport is believed to be of little importance to fertilization in ruminants (Mattner, 1973). Rapid sperm transport has also been demonstrated in the rabbit (Overstreet and Cooper, 1978; Overstreet and Tom, 1982) where it was concluded that most sperm involved in that phase of sperm transport were dead and were removed from the tract prior to ovulation thus eliminating them from any direct role in fertilization.

Recent evidence casts some doubt on the role of oxytocin as an agent of rapid sperm transport. The work of Schams et al. (1979; 1982), using radioimmunoassay for oxytocin in bovine plasma, revealed an oxytocin response in heifers exposed to various stimuli associated with natural or artificial mating but not in cows. These workers concluded that the absence of oxytocin response in cows indicated it had little physiological relevance to sperm transport and suggested other direct neural effects may be involved in mating-induced uterine contractions. Cooper et al. (1985) and Cooper and Foote (1986) suggested that uterine contractions in response to clitoral massage were neurally, not hormonally mediated, and probably a result of a reflex response.

Sustained sperm transport

The sustained phase of sperm transport occurs over a period of several hours and is characterized by a steady migration of viable sperm through the cervix and into the uterus and upper regions of the female genital tract. Sperm distribution in the female tract during sustained transport appears dependant upon sperm motility and uterine contractility. Sperm motility is necessary to penetrate mucus (Roark and Herman, 1950; Moghissi et al., 1964; Gaddum-Rosse, 1980a,b) and gain access to the cervical mucosa (Mattner, 1966). The sperm population that presumably participates in the sustained phase of sperm transport originates from that population located close to the cervical mucosa (Mattner, 1963a, 1966, 1968; Lightfoot and Restall, 1971). Nonviable sperm are excluded from access to the deep cervical mucosa or are eliminated from the tract by retrograde mucus flow (Mattner and Braden, 1969a; Lightfoot and Restall, 1971), presumably as they die or become immotile.

Uterine contractions during estrus are probably not involved in moving semen from the vagina into the cervix (Mattner, 1963a) but may have a role in transporting sperm across the uterus (Mattner, 1963b). Uterine contractions during estrus occur along the length of the uterine horn (Hawk, 1975; Ruckebusch and Bayard, 1975), progressing anteriorly from the uterine body early in estrus but changing direction of travel to become posteriorly directed late in estrus (Hawk, 1975).

The persistence of motile sperm in the cervix compared to other regions of the reproductive tract has been interpreted to indicate that the cervix functions as an embarkation point for sperm during the sustained transport phase (Mattner, 1963a, 1966, 1968). Sperm in the semen pool in the anterior vagina gain access to the cervix via the cervical mucus. As mucus is secreted, its flow is directed towards the vagina by the ciliated cells of the cervical epithelium whose kinocilia beat in that direction (Moghissi, 1972). The posteriorly- directed flow of mucus tends to orient the mucin glycoprotein macromolecules into long chains arranged roughly parallel to the longitudinal axis of the cervix, creating lines of strain along which sperm preferentially migrate (Tampion and Gibbons, 1962a; Mattner, 1966). *In vitro* studies have confirmed this observation, showing sperm orientation and migration parallel to the direction of stretch in drops of mucus drawn into threads, and more random sperm orientation and migration within unstretched drops of mucus (Tampion and Gibbons, 1962a; Moghissi et al., 1964; Gaddum-Rosse et al., 1980a,b). By following these paths of least resistance towards their points of origin in the cervical epithelium, sperm are directed into close proximity with the mucosa, away from the cervical canal (Mattner, 1966, 1968), and are found as aggregations of viable cells (Mattner, 1966, 1968) located in the crypts (Mattner, 1968) or groves (Mullins and Saacke, 1989) of the mucosa.

The quantitative aspects of sperm transport have been demonstrated in several species. In the bovine, Dobrowolski and Hafez (1970) charted the distribution of sperm in the female tract 1-, 8- and 24 hours after vaginal insemination. Their results, expressed as a percentage of total sperm recovered, showed a progressive decrease in vaginal sperm levels (from 77% to 58%) while uterine sperm levels progressively increased (from 1% to 15%) and

cervical sperm levels remained relatively constant (from 22% to 26%) with time post insemination. Sperm levels at the uterotubal junciton were low 1 hour post-insemination, increased at 8 hours and then did not change (.01%, .2% and .3%, respectively). Oviductal sperm levels were low at 1 hour post-insemination, increased sharply at 8 hours and then declined by 24 hours after insemination (.01%, .3% and .08% respectively). These results indicated that a progressive movement of sperm from the vagina to the uterus had occurred, with sperm passing through the cervix at a constant rate. The increase in levels of sperm at the uterotubal junction coincided with increased sperm levels in the uterus, but did not continue to rise appreciably after 8 hours post- insemination. Sperm levels in the uterotubal junction increased quite similarly to 8 hours post-insemination, however oviductal levels declined thereafter while uterotubal junction levels did not. In addition, El-Banna and Hafez (1970) showed that oviductal sperm numbers decreased between the 16th and 40th hours post-insemination, suggesting to Dobrowolski and Hafez (1970) that, when their data were combined with the former authors' data, it indicated that oviductal sperm numbers increased gradually and then declined.

Additional evidence concerning the timing of sperm arrival in the oviducts was supplied by Hunter and Wilmut (1983) and Wilmut and Hunter (1984). These authors demonstrated that fertilization rates and accessory sperm per egg were very low at 6 hours after natural mating but each progressively increased to maximal values at 12 hours. These results indicated that a population of sperm capable of reliably fertilizing the egg (based upon proportion of eggs fertilized) was not established in the oviduct until approximately 8-12 hours after mating. The authors indicated that the results supported the concept of a slow progression of viable sperm towards the oviducts in the sustained phase of sperm transport. The timing of sperm arrival in the oviducts as presented by Dobrowolski and Hafez (1970) corresponds with those of Hunter and Wilmut (1983) and Wilmut and Hunter (1984).

The quantitative effects of the sustained phase of sperm transport have been demonstrated in several other species, including the ovine (Mattner, 1963a,b; Quinlivan and

Robinson, 1969; Hunter et al., 1980, 1982), porcine (Hunter, 1975; Einarsson, 1980) and leporine (Overstreet et al., 1978).

Sperm Reservoirs

The establishment of sperm reservoirs in the cervix and uterotubal junction-lower isthmus area and possibly in the uterine glands coincides with the sustained phase of sperm transport and is characterized by the progressive and sequential accumulation of viable sperm in these locations. Limited evidence suggests that the uterine glands may possibly also constitute a sperm reservoir (Thibault and Wintenberger-Torres, 1967; Thibault, 1973; Thibault et al., 1975). In cattle and sheep, the cervix is believed to be the primary reservoir from which the continuous migration of sperm across the uterus originates (Mattner, 1963b, 1966, 1968). After vaginal semen deposition in cattle and sheep, evidence indicates that the cervix may function as a reservoir before moving across the uterus towards the oviducts (Mattner, 1963b, 1966, 1968). Sperm numbers in the cervix are high a short time before mating (Mattner, 1963a, 1973) and progressively decrease with time after mating as sperm numbers in more cranial portions of the tract increase (Quinlivan and Robinson, 1969; Mattner and Braden, 1969a; Dobrowolski and Hafez, 1970; El-Banna and Hafez, 1970; Mattner, 1973). In the bovine, Dobrowolski and Hafez (1970) found that of the total sperm recovered from the reproductive tract, the percentage recovered from the cervix remained relatively constant at 1-, 8- and 24 hours after vaginal semen deposition. This was interpreted as suggesting a constant rate of sperm movement through the cervix and/or the cervix regulating the movement of sperm across it. In the ewe, sperm numbers in the posterior and middle thirds of the cervix have been shown to decrease with time while levels in the anterior third remain relatively constant (Hawk, 1983; compiled from Hawk and Conley, 1975, Hawk and Cooper, 1977, Hawk et al., 1978) suggesting the anterior cervix as possibly the most important part of the cervical reservoir. The movement towards the uterus of sperm located close to the mucosa within the cervical folds may be expedited by following the predominantly sialomucin-filled grooves along the cervical mucosa to the uterus (Mullins and Saacke, 1982, 1989). Dead and non-

motile sperm are rapidly eliminated from the cervix, probably being unable to resist the posterior flow of cervical mucus (Mattner and Braden, 1969a).

Accumulations of sperm from the uterus may also populate the deeper cervical folds. After intrauterine insemination of ewes, Lightfoot and Restall (1971) demonstrated the colonization of the deep cervical mucosa when live sperm were used but not when using dead sperm. They concluded that sperm motility was a prerequisite for penetration between cervical folds but did not indicate the eventual fate of such sperm. In the cow, colonization of the cervical mucosa by 'backflow' sperm after intrauterine insemination and their subsequent reentry into the uterus has been speculated to occur (Larsson and Larsson, 1985; Larsson, 1988) but has not been demonstrated. The degree of retrograde colonization of the cervix is unknown, making the participation of the cervix as a sperm reservoir after intrauterine semen deposition as well as the contribution those sperm to fertility open to conjecture.

Conditions within the cervix appear favorable for sperm survival. Sperm have been reported to be able to survive in the bovine cervix for 2 to 3 days (Sergin et al., 1940; Laing, 1945b) and maintain their motility longer in mucus than in undiluted semen (Roark and Herman, 1950) with best survival in mucus from cows at or near estrus than in mucus from other stages of the estrous cycle (Olds and Van Demark, 1957c). Estrus cervical mucus contains elevated levels of reducing sugars (Olds and Van Demark, 1957a,b; Weed and Carrera, 1970; Smith, D. C. et al., 1970) that can be utilized by sperm as an energy source in aerobic or anaerobic metabolism (Mann, 1973). The deleterious effects of dilution on sperm survival do not affect sperm in mucus (Tampion and Gibbons, 1963b; Mattner, 1969c) possibly because the rapid diffusion of substances vital to sperm survival is inhibited by the glycoprotein structure of mucus (Mattner, 1969c; Katz and Singer, 1978). The reduced rate of sperm movement observed to occur in cervical mucus (Tampion and Gibbons, 1962b, 1963b) is not due to a motility inhibiting substance in mucus or the elevated pH of mucus (Tampion and Gibbons, 1962c, 1963a) but may result from increased drag exerted on sperm by their close proximity

to mucus glycoprotein macromolecules which reduces the thrust to drag ratio (Katz and Berger, 1980).

Motile sperm respond to the increased resistance such as encountered in mucus by changing their flagellar beat to one characterized by increased frequency and reduced amplitude and wavelength (Katz et al., 1978). It has been suggested that the change in sperm motility pattern may be due to a feedback on the flagellum from the increased viscosity of the external medium which results in an increased tendancy by the sperm to produce motion (Rikmenspoel et al., 1973). When swimming through mucus in close proximity to a surface parallel to their direction of travel, sperm have been reported to respond to the increased viscosity encountered there by changing their flagellar beat from a three-dimensional to a two-dimensional shape, and reducing the beat frequency (Katz et al., 1981a) which increases their velocity (Shulka et al., 1978; Katz et al., 1981a). The changes in the sperm's motility pattern in mucus enable it to swim through mucus whose viscosity both permits sperm penetration and travel but inhibits a rate of drainage that would exceed the rate of production (Tampion and Gibbons, 1963a).

The cervical secretion also serves to protect sperm from phagocytosis. Leukocytes, primarily of uterine origin (Mattner, 1968, 1969a), are found in larger numbers in the central than in the more peripheral areas of the cervical lumen, having been directed there presumably by the alignment of mucus glycoprotein macromolecules and greater rate of mucus flow in that area (Mattner, 1968, 1973). Sperm actively migrating in a cranial direction in close proximity to the deep cervical mucosa (Mattner, 1969a) or between mucosal folds (Lightfoot and Restall, 1971) are spatially separated by the posteriorly flowing mucus from the more centrally located leukocytes (Mattner, 1969a; Lightfoot and Restall, 1971). The physical separation of sperm from leukocytes by mucus (Mattner, 1969a; Lightfoot and Restall, 1971) and the tendency of leukocytes to not migrate towards sperm in mucus (Mattner, 1969b) appear to function together in protecting sperm in the cervical reservoir.

Although the uterine glands have been proposed as a possible sperm reservoir (Robinson, 1975) there is scant evidence to support that proposition. Sperm have been found

in the uterine glands of the cow (Thibault, 1973) and ewe (Thibault and Wintenberger-Torres, 1967) in increasing numbers to 18 hours after mating, whereupon they progressively disappear (Thibault et al., 1975). Reports of sperm survival in bovine uterine fluid for up to 7 hours *in vitro* (Olds and Van Demark, 1957c), and a differential maintenance of motility in rabbit sperm cocultured with and without rabbit endometrial cells (Boice and Duby, 1987) suggest some sustentacular effect of the endometrium on sperm. These observations, coupled with collateral evidence in lower vertebrates (Austin, 1960; Thibault, 1973) suggest that the uterine glands may function as a sperm storage site; however, more intensive investigation appears to be indicated.

The existence of a sperm reservoir in the uterotubal junction - lower isthmus area was suggested by the results of studies that showed an increase and maintenance in oviducal sperm numbers with time after mating (Quinlivan and Robinson, 1969; Dobrowolski and Hafez, 1970; El Banna and Hafez, 1970). Thibault (1973) reported a progressive increase in sperm numbers at the bovine utero tubal junction from 2 to 18 hours after mating that was accompanied by increased sperm numbers in the lower isthmus region from 8 to 18 hours after mating, strongly indicating a storage function of these areas. Thibault et al. (1975) made similar observations, noting live sperm located within the folds of the bovine uterotubal junction and dead ones in the lumen. They suggested that the uterotubal junction acted as a storage container while the lower isthmus acted as a filter that restricted the number of sperm reaching the upper isthmic areas.

Overstreet et al. (1978) demonstrated the importance of the lower isthmus rather than the uterotubal junction as a sperm reservoir in the rabbit. They found that the utero-tubal junction functioned to reduce the number of sperm entering the oviduct while the lower isthmus restricted the further cranial migration of sperm until near the time of ovulation. Sperm numbers in the lower isthmus were found to progressively increase as uterine sperm numbers increased with time after mating. Although sperm viability was initially high in the lower isthmus, levels of nonviable sperm increased by 4 hours after mating and remained level thereafter. Similarly, flagellation by viable sperm was reduced from initially elevated

levels. Around the time of ovulation, sperm numbers in the upper isthmus increased, exhibiting a greater level of viability and flagellatory vigor than lower isthmus sperm. The authors suggested that the depressed motility of lower isthmus sperm may have had the greatest effect on their sequestration in that region until the periovulatory time period. The results also suggested that the lower isthmus constituted a viability barrier that appeared to allow only a highly viable sperm population into the upper isthmus to increasing degrees in the 6- to 12 hour time period after mating.

The work of Hunter and Wilmut (1983, 1984) and Wilmut and Hunter (1984) in the bovine lent support to this suggestion by showing that a sperm population competent to effect fertilization was established in the lower oviduct over a period of at least 6 hours to more than 12 hours after mating. By ligating the caudal isthmus immediately adjacent to the uterotubal junction at intervals from 6 to 36 hours after mating, it was demonstrated that fertilization did not occur before 6 hours after mating and that the proportion of eggs fertilized and the number of accessory sperm per fertilized egg showed an increasing trend from 8 to 12 hours after mating (Hunter and Wilmut, 1983; Wilmut and Hunter, 1984). It was observed that fertilizing sperm were held in the caudal isthmic area for 18 hours or more until ovulation was imminent, whereupon there occurred a progressive displacement of viable sperm toward the site of fertilization (Hunter and Wilmut, 1984). Similar observations have been reported in the ewe (Hunter et al., 1980, 1982a), sow (Hunter, 1981) and mouse (Suarez, 1987; Suarez and Osman, 1987). The sequestration of sperm until the time coinciding with ovulation suggests a close interaction between sperm migration and oviductal environment (Hunter, 1985) and may be under local control of ovarian follicular hormones (Hunter, 1986). That the oviductal isthmus has the cauda epididymis as its embryonic analog (caudal Mullerian duct vs. caudal Wolffian duct) should not be ignored when contemplating the storage function of the lower oviduct (Hunter and Nichol, 1983; Suarez, 1987).

Sperm Loss

Recovery of low proportions of inseminated sperm from the female reproductive tract soon after insemination indicate that the majority of sperm deposited in the female quickly disappear. Dobrowolski and Hafez (1970) recovered from 0.7% to 16.7% of the vaginally-deposited sperm deposited in heifers across all time post-insemination, averaging 13.4% recovered 1 hour after insemination, 3.8% at 8 hours and 0.9% at 24 hours. El Banna and Hafez (1970) recovered 1.6% and .03% of the vaginally deposited sperm from cows bred 16 and 40 hours, respectively, before recovery. After intrauterine insemination, Suga and Higaki (1971; cited in Hawk, 1987), recovered sperm from the reproductive tract of dairy cows at intervals of 3 to 30 minutes, 30 to 60 minutes, 1 to 2 hours, 2 to 3 hours and 3 to 5 hours later. Proportions of inseminated sperm recovered were greatest in the first time period (11.7%), were reduced to 1.4% in each of the next two times post insemination and increased to 6.7% and 7.3% in the last two sperm recovery times (Adapted from Suga and Higaki, 1971 as cited in Hawk, 1987). Larsson and Larsson (1985) recovered 14.6% and 0.6% of inseminated sperm from artificially inseminated heifers at 2 hours and 12 hours post insemination, respectively, while Mitchell et al. (1985), in two experiments, reported 6.3% and 6.5% of sperm deposited in the uterus remained in the genital tract 12 hours after insemination. These findings indicate that much of the sperm deposited in the female is rapidly lost and that site of semen deposition appears to have little effect on the rate and magnitude of that loss. Similar degrees of sperm loss have been reported in the ewe (Quinlivan and Robinson, 1969; Hawk and Conley, 1975) sow (First et al., 1968) and doe rabbit (Morton and Glover, 1974a). The major routes of sperm loss from the female appear to be retrograde removal via cervical mucus, phagocytosis by polymorphonuclear leukocytes, and passage into the body cavity via the oviducts after completely traversing the tract.

Retrograde Loss

The results of quantitative sperm transport studies using natural- and artificial mating indicate that most sperm are lost in expelled mucus after mating. Dobrowolski and Hafez (1970) noted that most of sperm recovered 1-, 8- and 24 hours after vaginal semen deposition was of vaginal origin and suggested that expulsion of sperm in vaginal mucus after copulation resulted in reduced sperm numbers at the oviducts. Suga and Higaki (1971; cited in Mitchell et al., 1985 and in Hawk, 1987) found that the majority of sperm recovered from cows 1 or more hours post-insemination were found in the vagina after intrauterine semen deposition. Larsson (1984) reported 98.5% and 73.2% of sperm recovered from heifers inseminated in the uterine corpus were found posterior to the site of semen deposition at 2 and 12 hours post insemination, respectively, and suggested sperm expulsion in mucus as partially responsible for reducing sperm numbers. Larsson and Larsson (1985) found the majority of recovered sperm in the vaginal mucus of heifers 2- and 12 hours after intrauterine insemination, indicating a considerable backflow of sperm through the cervix had occurred during the first 12 hours after insemination. Mitchell et al. (1985) conducted an experiment to quantitatively account for the relative retention and loss of sperm following insemination when a significant retrograde movement of sperm was noted following intrauterine insemination in a preceding experiment. In the 12 hours following insemination, 73% of inseminated sperm were accounted for, with 65% recovered in the expelled mucus. When only sperm in mucus were considered, 79% and 92% of those sperm had exited by 6- and 8 hours post insemination, respectively. Of the sperm retained in the genital tract 12 hours after insemination, 89% were found in the vagina. This study provided definitive proof that the retrograde removal of sperm in mucus was the major route of sperm loss from the genital tract of the artificially inseminated cow.

Phagocytosis

While retrograde removal of sperm from the female genital tract has been credited as a major route of sperm loss, phagocytosis has also been acknowledged for some reduction

in sperm numbers. Polymorphonuclear leukocytes are found in all regions of the female genitalia, with large populations in the uterine endometrium and few in the cervical mucosa (Mattner, 1968). Low levels of leukocytes are found in the female tract at all times; however numbers increase with the approach of estrus and decrease shortly thereafter, notably few being of cervical origin (Roark and Herman, 1950). It has been demonstrated that the female reproductive tract, particularly the uterus, exhibits a strong defensive ability against infection during estrus, but which appears reduced during the luteal phase (Black et al., 1954; Olds and Van Demark, 1957a). Circulating and endometrial levels of leukocytes increase around the time of estrus, presumably augmenting the uterine defensive capabilities (Hawk, 1971). Under progesterone influence, uterine leukocyte phagocytic activity is inhibited by some factor in the luteal phase uterine millieu (Killingbeck and Lamming, 1963), while under estrogenic influence that inhibition is removed (Lamming, 1961) and greater leukocytic response ensues (Hawk, 1971). Recently, however, Roth et al. (1982, 1983) reported enhanced leukocytic activity following induction of high serum levels of both estradiol and progesterone in the bovine. While phagocytic activity of uterine leukocytes is characteristically reduced compared to that of leukocytes in the circulation (Romaniukowa, 1984), passage through bovine reproductive tract epithelium *in vitro* has been demonstrated to enhance their phagocytic abilities (Targowski, 1986).

In a study conducted to examine sperm transport in heifers, Howe and Black (1963) reported the presence of large numbers of polymorphonuclear leukocytes in samples taken from the vagina and uterus after vaginal semen deposition. While low numbers of leukocytes were present in samples taken prior to insemination, leukocytic infiltration was observed to increase between 4 and 8 hours after insemination reaching a maximum by 8- to 16 hours after which there was a decline. They concluded that the presence of semen stimulated this response since the extender did not when used alone, and bacterial activity within the extended semen was negative. In hematoxylin-stained smears prepared from vaginal contents, active phagocytosis of sperm was noted, particularly in samples obtained 4 to 8 hours after insemination. The degree of phagocytosis observed at any one time post-insemination indi-

cated to these authors that leukocytes did not play a major role in the disappearance of sperm from the tract.

Mattner (1968) reported that the presence of sperm in the genital tract resulted in increased leukocyte numbers in the uterine and cervical lumens. Nineteen to 22 hours after natural mating, leukocytes, some containing sperm, were found in all parts of the genital tract, with greater numbers present in the uterine lumen than in the cervical lumen. Although leukocyte numbers in the cervical lumen were related to those in the uterine lumen, a similar relationship could not be established between leukocyte levels in cervical stroma and cervical lumen or between leukocyte levels in the endometrium and the uterine lumen. While endometrial leukocytes were abundant, few leukocytes could be found in the cervical stroma. Numerous leukocytes were observed in mucus flushed from the cervical canal; however, few were found in mucus retained between cervical villi. These findings led Mattner (1968) to conclude that cervical leukocytes were primarily of uterine origin, and were conveyed to the cervical canal by the posterior flow of mucus. Mattner (1968) proposed that spatial separation by mucus of leukocytes in the more central cervical canal from the more peripherally situated sperm would protect those sperm by limiting contact with the phagocytes. Mattner (1969b) later reported that the physical structure of cervical mucus appeared to prevent migration of leukocytes in that secretion since leukocytic migration and phagocytosis appeared unimpeded in liquified (macerated) mucus. Mitchell et al. (1985) found that leukocyte concentration in mucus samples recovered at 2 hour intervals for 12 hours following intrauterine semen deposition did not differ among the six sample times. Sperm phagocytosis was observed by these authors but not quantified. They proposed, none the less, that many of the sperm in the unrecovered portion of the inseminate had been phagocytized by leukocytes.

It is apparent that a leukocytic response to sperm occurs in the female reproductive tract and that phagocytosis of some of those sperm occurs. It is not known, however, to what extent leukocytic phagocytosis contributes to the reduction of sperm numbers in the female or whether leukocytes are attracted preferentially to some sperm and not others (Cohen, 1984). Further studies in this area are warranted.

Passage Into Body Cavity

A third route of sperm loss from the reproductive tract is by complete traversal of the tract and exit into the peritoneal cavity via the infundibulum. With the phases of sperm transport in mind, it would seem reasonable that sperm would be lost via this route as a result of rapid sperm transport which involves relatively few sperm or by migration beyond the site of fertilization after release from the lower isthmic reservoir established during the sustained phase of sperm transport.

Sperm have been recovered from the ovarian aspect of the oviduct in the cow (Beshlebnov, 1938; Van Demark and Hays, 1954, 1955; Van Demark and Moeller, 1951; Larsson and Larsson, 1985) the ewe (Quinlan, Maré and Roux, 1932; Green and Winters, 1936; Phillips and Andrews, 1937; Schott and Phillips, 1941; Starke, 1949; Mattner and Braden, 1963), rabbit (Parker, 1930; Overstreet and Cooper, 1978) and human (Settlage et al., 1973). The appearance of sperm in the anterior oviduct or infundibulum does not prove passage into the body cavity. However, direct evidence of sperm passage into the body cavity after insemination was reported by Larsson (1986c) in heifers. After a distal segment of one oviduct in each heifer was resected between ligatures placed 2-3 cm apart, estrus heifers were artificially inseminated. Sperm were found above the point of ligation and resection in all heifers 2 hours after insemination, indicating sperm exited the tract via the infundibulum of the patent oviduct (contralateral to the ligated and resected oviduct) traversed the peritoneal cavity and entered the ligated and resected oviduct via its infundibulum. Although sperm numbers recovered above the point of resection were low, it was suggested more sperm would have been recovered there had ovulation occurred and with increased time between insemination and recovery. Similar evidence has been reported in the human (Brown et al., 1987) demonstrating that ectopic pregnancy can result from sperm passage from one oviduct, via transperitoneal migration to the contralateral oviduct detached from the uterus. That pregnancy occurred indicates the sperm were of fertilizing quality.

Sperm loss from the female reproductive tract via this route has neither been quantitated nor qualified but is believed to be a small proportion of the inseminated sperm

(Hawk, 1983). The limited evidence allows for much speculation concerning the roles of rapid and sustained sperm transport, the quality and number of sperm thus removed and the effect on fertility this mode of sperm loss incurs.

Semen Quality and Fertility

To satisfy the fertility requirements of the female, the semen produced by the bull must meet certain quantitative and qualitative requirements. In predicting the bull's fertility level, Laing (1945a) proposed that semen volume, sperm density and thus total number of sperm (quantitative criteria) in addition to sperm viability and morphology (qualitative criteria) must be considered. Diagnosis of the male's level of fertility was necessary, he stated, in order to eliminate sterile bulls and bulls of reduced fertility and thus increase the reproductive efficiency of cattle. Semen quality tests have been devised to subjectively and objectively evaluate the fertility-related viability and morphological traits of a semen sample and have been reviewed by Bratton et al. (1956), Elliott (1978a,b), Sullivan (1978), Pace (1980), Saacke (1970, 1982, 1984), Saacke et al. (1980), Garner (1984) and Uwland (1984). Technical and biological factors have obscured the relationships between fertility and semen quality traits as judged by laboratory evaluation (Saacke, 1982). Many laboratory tests of semen quality are not highly repeatable (Graham, 1978), measures of fertility lack accuracy and low variation in fertility makes the interpretation of fertility relationships with semen quality difficult (Oltenacu and Foote, 1976; Linford et al., 1976; Pace, 1980). Some biological aspects that may impinge upon the relationship between semen quality and fertility are the interaction of sperm quantity with sperm quality, selective sperm transport and persistence in the female genital tract, and differential abilities of sperm to fertilize oocytes and/or sustain embryo development (Saacke, 1982).

It has long been recognized that although only one sperm is required to fertilize the egg, unless adequate numbers of sperm are deposited in the female reduced fertility occurs

(Laing, 1945a). In a study of the relationship between fertility and semen characteristics, Milovanov (1937) proposed that relationship be represented by the formula $F = K^m\sqrt{n}$ where F represented percent fertility, n the number of sperm and K a constant found empirically by substituting a value of m to make it constant with known values for F and n obtained from field trials, the value for K could be ascertained by rearranging the equation and solving for K^m. For sheep, K was found to be nearly constant (ranging between 1.68 and 1.70) when $m=5$, while for cattle, pigs and rabbits, $m=3$ yielded a constant K. It was noted that other factors could influence fertility, namely site of insemination, sperm motility, resistance to dilution, storage conditions and numbers and times of inseminations, whose effects could be used to modify K in the equation. This formula yielded a curvilinear relationship where fertility initially increased fairly directly with increased values of n to a point beyond which fertility increased at a reduced rate. At that point of diminishing returns, Milovanov (1937) suggested that further increases in F could be attained by increasing the value of K by improving the factors noted above, some of which are recognized to affect semen quality.

Salisbury and Van Demark (1961) proposed a similar model for the relationship between the numbers of sperm having a certain characteristic and the level of fertility. Their asymptotic model predicted that for a female population of given fertility, maximum fertility of those females could be attained by increasing the total number of sperm in an inseminate having a given sperm characteristic to a level (threshold value) beyond which any further increases in sperm numbers would not result in a further increase in the fertility of the population. The male would impose constraints upon fertility if suboptimal numbers of sperm were delivered. The female would dictate the upper limit of fertility when optimal numbers of fit sperm were delivered. Semen viability traits that have been shown to conform to this model are sperm motility (Sullivan and Elliott, 1968; Pace et al., 1981), acrosomal integrity, swelling response to hypotonicity and number of sperm passing through Sephandex columns (Pace et al., 1981).

For maximum fertility, many more sperm are required by the female than are simply needed to obtain fertility. The removal of large numbers of sperm from the female genital tract

has been well established and undoubtedly involves those sperm not participating in fertilization. As an explanation for the drastic reduction in male gamete numbers that occurs in the female tract post-insemination, Cohen and McNaughton (1974) proposed that either active or passive selection occurs in the female reproductive tract which results in a viable population of sperm more "fit" to accomplish the task of fertilization. Given two gamete populations of equal size, one containing a higher proportion of unfit cells than the other, their model would predict that fewer sperm would remain post-selection from the population containing the higher proportion of unfit sperm than from the other population.

A combination of the Salisbury and Van Demark (1961) and Cohen and McNaughton (1974) models could explain the fertility reduction that occurs with increased levels of unfit sperm in the ejaculate/inseminate. The combination of the models would predict that, as the proportion of unfit sperm increased in the inseminate/ejaculate, the proportion of inseminated sperm selected out by the female tract would also be increased. The result in the female tract after selection would be decreased total sperm numbers (due to selection) available for fertilization. This decrease in total sperm numbers could result in sub-threshold levels of sperm and thus could reduce fertility.

Ejaculated semen contains a population of sperm that is generally heterogeneous in nature across a wide range of traits including motility, maturity and morphology (Hunter, 1980). Several early reports (Williams, 1920a,b; 1922; Savage, 1924a,b), citing the incidence of reduced fertility in herds using bulls that produced sperm of abnormal appearance, can be regarded as initiating the study of the relationship between fertility and sperm abnormalities. Current morphological evaluation of bovine sperm evolved from clinical observations reported by Williams and Savage (1925, 1927) and Lagerlöff (1934) which described the incidence of abnormal sperm production in sterile and subfertile bulls. The former authors examined the semen from over 400 bulls, noting that reproductive inefficiency due to clinical lesions of the male genital tract occurred in between 10-30% of the cases and that approximately 11% demonstrated changes in the spermatozoa. These authors noted that the length of sperm heads from bulls of good fertility were notably uniform, that no more than 17% abnormal

sperm was exhibited in the ejaculates of the "...highly efficient..." bulls and that the types of sperm abnormalities appeared to differ in the severity of their effects on fertility, and thus could, depending upon the type of abnormality, be permissible to varying degrees in the ejaculate. Taking the observations of Williams and Savage (1925, 1927) on sperm head length a step farther, Savage et al. (1927) analyzed the head lengths of sperm obtained from semen samples of bulls exhibiting various levels of fertility. They found that the coefficients of variation (CV) for sperm head length of bulls having good fertility (few repeat services per conception) were distributed symmetrically about a mean CV, while slight asymmetry of distribution about a somewhat larger mean CV was evident for bulls of fair fertility (up to 1.5 services per conception) and an erratic, skewed distribution about a still higher mean CV occurred for bulls of poor or very poor fertility (many repeat services required, high abortion rate, genital lesions apparent). These authors concluded that if the curves describing the distributions were abnormal (i.e. skewed), then dependable identification of unsound sires was assured, while a normal curve (i.e. symmetrical) did not necessarily indicate normal fertility.

Lagerloff (1934) described the microscopic appearance of normal and abnormal sperm from over 2000 bulls examined between 1928 and 1932. More than 23% of the bulls examined showed reduced fertility, and from their semen he reported on the morphology of abnormal sperm heads, midpieces and tails, and the presence and location of cytoplasmic droplets. In summarizing those and subsequent findings, Lagerlöff (1936) laid out criteria for judgement of reduced fertility regarding semen examination. He noted that while abnormal sperm averaged 10-12% and did not exceed 17-18% in bulls of normal fertility, reduced fertility could be expected when levels of pathological sperm exceeded 20%. The occurrence of abnormal sperm was linked to pathological changes in the testes, he felt, and stated that the pathological changes that resulted in the formation of abnormal sperm may also have adversely affected sperm other than those showing morphological abnormalities.

Other authors corroborated the findings of Williams and Savage (1925, 1927) and Lagerlöff (1934, 1936) and underscored the necessity of a thorough physical examination of the

bull combined with detailed quantitative and qualitative evaluation of the semen to best assess a bull's potential fertility (Donham et al., 1931; Addis, 1937; Generales, 1938; Sciuchetti, 1938; Herman and Swanson, 1941; Laing, 1945a; Rollinson, 1951a,b,c). Although it was noted by several authors that a relationship appeared to exist between levels of abnormal sperm and the fertility of the bull, some authors were unable to demonstrate significant correlations with fertility (Donham et al., 1931; Swanson and Herman, 1941; Laing, 1945a).

In studying the effects of season on spermatogenic activity as judged by fertility and semen quality traits, Mercier and Salisbury (1946) noted a low but significant negative correlation between the proportion of morphologically abnormal sperm in semen and the fertility of that semen. Sixty day non return rates averaged 58% for over 20,000 inseminations from 328 ejaculates collected an average of every eleven days for a year. Although only 10 bulls were studied during the year long trial, the semen exhibited marked variation in quality among bulls and among months of collection. Similar results were reported by Cupps et al. (1953) demonstrating significant negative correlations between levels of abnormal sperm and fertility in a study employing three inbred bulls that produced low quality semen and two bulls whose semen was normal. Semen quality characteristics were highly variable as was the 30-60 day non-return rate (87% to 55%) for more than 1,000 inseminations.

Blom (1973) proposed that morphological abnormalities of bull sperm be classified as either major (abnormalities correlated to impared fertility) or minor (other deviating forms of minor importance). Although this classification approaches sperm morphology evaluation from an impact-on-fertility standpoint, it results in a confusing array of head, midpiece, tail and protoplasmic droplet abnormalities within each category. A third category for non-gamete cells, such as epithelial cells and leukocytes, was included. Blom (1983) updated the semen evaluation techniques while retaining the original spermiogram format, and included step by step instructions for the morphological evaluation of semen.

Saacke and White (1972) suggested primary, secondary and tertiary as designations of sperm abnormalities for aberrant head and acrosome morphology, presence and location of protoplasmic droplet, and tail deformation, respectively. Their investigation of fertility re-

lationships with sperm motility, acrosome integrity and sperm morphology revealed among male correlations of fertility with sperm motility and acrosome integrity to be significant and positive, while relationships for primary abnormalities and secondary abnormalities with fertility were negative and significant. Tertiary abnormalities (deformities of the tail) were not correlated with fertility. Bulls of above average fertility appeared to produce lower levels of abnormal sperm within narrower ranges than did bulls of below average fertility. Although correlations with fertility became non-significant when variation due to differences among bulls was removed, these authors cautioned that semen quality evaluation of ejaculates within bulls should be considered meaningful in identification of a bull's ejaculates for culling. The loss of significance in this case was thought to be due to the low variation of semen quality among ejaculates within bulls (Saacke, 1982), which could change with season (Sekoni and Gustafsson, 1987) or illness (Chenoweth and Burgess, 1972; Foster et al., 1980).

Fertility reductions related to increased levels of abnormal sperm presented to the female may be a result of a number of factors. Abnormal sperm may be victims of discrimination in transport or reduced viability, leaving the females threshold requirement unfulfilled. In addition, abnormal sperm may also be preferentially removed by retrograde flow or phagocytosis to yield the same result. It is also possible that fertility reductions related to abnormal sperm occur due to impaired ability to fertilize the oocyte. Although structurally abnormal sperm have been reported to accomplish fertilization (Smith, D. M. et al., 1970; Krzanowska and Lorenc, 1983), they appear to do so in lower frequencies than normally shaped sperm. Abnormal sperm bind to the zona pellucida in reduced frequencies and at different angles than do normal sperm (Kot and Handel, 1987), and exhibit reduced rates of penetration of the zona pellucida compared to normal sperm (Marsh et al., 1987), suggesting a mechanism behind reduced fertilization. Having accomplished fertilization, abnormal sperm may be unable to sustain normal embryonic development.

In a review concerning the male contribution to early embryonic mortality, Mattei (1984) described the high rate of human early spontaneous abortions as due in large part to zygotic deformities. It was concluded that the human male was a significant contributor to

chromosomal and structural anomalies leading to early embryonic death. Comparisons with domestic livestock species showed some similarity in frequency of chromosomal abnormalities in those species, many of which resulted in reproductive failure.

Courot and Colas (1986) noted that the male's contribution to fertility involved fertilization of oocytes such that normal development and survival of the conceptus was promoted. In bulls, it has been demonstrated that lower embryonic death is associated with the use of high fertility bulls than for bulls of lower fertility (Kidder et al., 1954; Erb and Flerchinger, 1954; Bearden et al., 1956). Higher pregnancy rates have been reported when bulls are selected for use on the basis of high levels of normal sperm (Wiltbank and Parish, 1986) while reduced fertility follows the use of bulls exhibiting high levels of specific sperm abnormalities (Cran and Dott, 1976; Blom, 1980; Heath and Ott, 1982; Miller et al., 1982; Lorton et al., 1983; Czaker, and Mayer, 1984; Barth, 1986). Reduced semen quality in bulls has been related to such factors as season (Sekoni and Gustafsson, 1987), illness (Chenoweth and Burgess, 1972; Foster et al., 1980), mineral deficiency (Heimann et al., 1982), environmental temperature (Ax et al., 1987; Parkinson, 1987), testicular degeneration (Rao and Bane, 1985), disturbed spermiogenesis (Bane and Nicander, 1965; Williams, 1987), chromosomal abnormalities (Koefoed-Johnsen et al., 1980) and genetics (Roslanowski, 1969, 1970). Male-related embryonic death has been linked to some of the factors that also result in reduced semen quality such as heat stress (Brockhausen et al., 1978; Setchell et al., 1988), season (Colas, 1983) immature sperm (Courot and Colas, 1986) and aged sperm (Salisbury and Hart, 1970; Koefoed-Johnsen et al., 1971). The DNA content of abnormal bull sperm does not differ from that of normal bull sperm (Gledhill, 1966) but may be abnormally condensed (Gledhill et al., 1971). Nevertheless, abmormal sperm morphology may contribute to subfertility/sterility by virtue of selective exclusion from the fertilization process or by their incompetence in sustaining the embryo following fertilization.

Transport of Abnormal Sperm

The fertility reduction associated with increased levels of abnormal sperm may be a result of impaired transport of those sperm. Two structures of the female genitalia commonly recognized as barriers to sperm transport are the cervix and uterotubal junction. In those species where intracervical or post-cervical semen deposition occurs, the uterotubal junction is considered the primary physical barrier to sperm transport and acts as a sperm reservoir and also to regulate the passage of sperm across it (Blandau, 1945; Leonard and Perlman, 1949; Rigby, 1964; Krzanowska, 1974; Hunter, 1975a,b; Pursel et al., 1978; Einarsson, 1985). In those species where vaginal semen deposition occurs naturally, the cervix fulfills a role similar to that of the uterotubal junction in the pig, mare, mouse and rat.

That the bovine cervix influences the transport of vaginally deposited sperm has been well established. The unique anatomy and physiology of the cervix and its secretion affect the transport of sperm by providing i) a means for their entry into the organ, ii) an environment conducive to their survival, iii) a structure promotive to their passage, iv) a mechanism for removal of nonviable cells and v) a haven from leukocytic phagocytosis. An increasing amount of evidence suggests that the cervix also functions to regulate the passage of sperm based upon their morphology. Following vaginal deposition of semen and subsequent cervical passage, uterine sperm may be a selected population due to the nature of the cervix, cervical mucus, the uterus itself, differences among intrinsic sperm qualities or any combination of these factors.

Using semen from bulls producing normal semen profiles, Roslanowski and Koefoed-Johnsen (1971) observed *in vitro* that the percentage of morphological normal sperm penetrating bovine estrus mucus was significantly greater than that in the live population of sperm before penetration. This difference was attributed to a sorting mechanism exerted by the cervical mucus. A lower proportion of dead sperm was noted for those of normal morphology than for abnormal sperm, suggesting preferential death of abnormal sperm before or upon

ejaculation. Koefoed-Johnsen (1972) conducted a similar experiment using semen from four bulls producing high levels of morphologically abnormal sperm, particularly abnormal tails. Two of the bulls exhibited normal fertility and were in use as artificial insemination (A.I.) sires. After mucus penetration the proportion of total abnormal sperm and proportions of individual types of abnormal sperm were significantly reduced. The magnitude of abnormality reduction differed among bulls and by type of abnormality, with the proportion of head, protoplasmic droplet and tail abnormalities decreasing 75%, 92% and 88%, respectively. Abnormalities of the tail and protoplasmic droplet comprised 72% and 22%, respectively, of the total abnormalities reported. Self selection due to impaired motility of sperm with abnormal tails or sperm immaturity could have accounted for the reduction ascribed to mucus selection. It is not known to what extent multiple abnormalities were observed on the same sperm, such as an abnormal head coupled to an abnormal tail, leaving open to question the possibility of reduction in other abnormalities linked to reduction due to tail abnormalities.

In vivo, and 12 hours following intrauterine insemination, Mitchell et al. (1985) noted a reduction in the proportion of sperm with tapered heads and sperm with a knobbed acrosome defect recovered from the bovine uterus, cervix and vagina as compared to that of the inseminate. These authors speculated that a reduced motility of the sperm with head abnormalities may have affected retention of those sperm in the female genital tract. Although the proportion of sperm recovered having secondary and tertiary abnormalities was reduced compared to the inseminate, mechanistic interpretation of the data was complicated by the low frequency with which those abnormalities occurred in the inseminates.

Larsson (1988) reported reduced frequencies of sperm with abnormal heads recovered from the cervix, uterus and oviducts as compared to the inseminate 2 hours after intrauterine insemination with frozen semen. Sperm exhibiting severe head abnormalities were more often recovered from the vagina and vaginal mucus than other parts of the tract, while sperm with neck implantation or tail defects (but normal heads) were more often recovered from the vagina and the uterus than other parts of the tract. Of the abnormal sperm found in the tract, the greatest number occurred in the vagina indicating exclusion of those morphological forms.

It was suggested that a cervical or uterine mechanism selected against sperm with abnormal head morphology and impaired motility associated with neck and midpiece defects may have acted separately or in combination to eliminate those sperm.

The apparent preferential elimination of abnormal sperm from the cow reproductive tract may be related to the morphology and/or viability of those sperm. While *in vitro* tests of bovine sperm migration in various media have demonstrated the close relationship of sperm migrating abilities with levels of live sperm and motile sperm (Parrish and Foote, 1987), and the results of mucus penetration tests (Roslanowski and Koefoed-Johnsen, 1971; Koefoed-Johnsen, 1972) also imply a motility effect, the exclusion based upon head morphology alone has not been addressed. Dresdner and Katz (1981) investigated relationships of sperm head morphology and flagellar beat characteristics with swimming velocity, power output and stirring of nearby fluid (hydrodynamic characteristics) and concluded that small differences in geometric parameters of morphology and movement could produce large differences in hydrodynamic characters. The results reported by Katz et al. (1981) supported the findings of Dresdner and Katz (1981) by demonstrating that morphologically normal sperm swam faster and straighter with higher flagellar beat frequency than sperm with amorphous, tapered, pyriform, enlarged or pin heads. Similarly, Gaddum-Rosse et al. (1980a) observed negligible penetration of heterologous sperm (rabbit, guinea pig and mouse) into human midcycle- and bovine estrual cervical mucus. These authors suggested that the impaired penetration may have been due to either dissimilarity in motility pattern of heterologous vs homologous sperm, which resulted in inefficient movement, or head size differences which impeded access. Headless, but motile guinea pig sperm tails easily penetrated the mucus where intact sperm were excluded.

Using the rabbit, Mortimer (1977) found a significant reduction in the numbers of abnormal sperm in the cervix and uterus, as compared to the ejaculate, 6 hours after breeding does to a buck that produced high levels of diploid (giant head) sperm. Although the proportion of nonviable diploid sperm was higher than nonviable normal sperm in the ejaculate, *in vitro* incubation of ejaculate samples did not show differential duration of viability for diploid

vs normal sperm. It was concluded that differences between inseminate and uterine numbers of diploid sperm and between the inseminate and oviducts for numbers of diploid sperm were due to the barrier the cervix and uterotubal junction posed to the impaired motility of these abnormal sperm. An alternate explanation could lie in the observations of Lorton and First (1977), who concluded that failure of head to head agglutinated sperm (non-Rouleau pattern) to migrate through bovine mucus *in vitro* was not due to impaired motility, but possibly due to their inability to fit into and pass through the mucus glycoprotein framework.

Perry et al. (1977) demonstrated the exclusion of human abnormal sperm *in vitro* from columns of human cervical mucus. Two populations of sperm, one designated 'normal' (<20% morphologically abnormal sperm), the other designated 'abnormal' (>35% morphologically abnormal sperm), were allowed to migrate up separate columns of good quality human cervical mucus. For both the normal and abnormal semen samples, the number of abnormal sperm that had migrated 3 cm up the column was significantly reduced compared to the number that had migrated 1 cm up the column. The number of normal sperm that had migrated 3 cm up the column did not differ from the number that had penetrated to 1 cm in each type of semen sample. Bergman et al. (1981) reported similar results in a comparison of human sperm penetrability into human and bovine cervical mucus columns. In this study, a highly significant selection of sperm was demonstrated, with exclusion of sperm having giant heads, amorphous heads or neck pathology, moderate penetration by sperm having tapering-, micro- or pin heads, and good penetration by normal forms. Other *in vitro* studies of mucus penetration by human sperm ascribe the reduction in abnormal sperm after mucus passage to impaired motility, not abnormal morphology (Mortimer et al., 1982; Barros et al., 1984; Jeulin et al., 1985; Mortimer et al., 1986a,b). The results of *in vivo* observations concerning the effects of cervical passage on sperm morphology tend to support the *in vitro* results (Insler et al., 1980; Ragni et al., 1985; Gonzales and Jezequel, 1985).

In species where intracervical semen deposition naturally occurs, the role of the uterotubal junction appears similar to that of the cervix in ruminants (Bedford, 1970). Krzanowska (1974) demonstrated the ability of the mouse uterotubal junction to act as a se-

lective barrier to sperm with morphologically abnormal heads, particularly those severely deformed. Female mice were inseminated naturally and artificially using males that produced high levels of abnormal sperm. The proportion of sperm with abnormal heads recovered from the oviducts were positively related to, and markedly lower than that in the population recovered from the uterus. Sperm with more subtle head abnormalities were less efficiently excluded from the oviductal population than were sperm with severe head abnormalities. The basis for exclusion did not appear to be due to impaired motility of the abnormal sperm, the time of insemination in relation to ovulation or unsuccessful competition of abnormal sperm with normal sperm in the uterus for access to the uterotubal junction. De Boer et al. (1976) observed selection against abnormally shaped sperm at the mouse uterotubal junction but tied selection against abnormal morphology to abnormal motility. The head morphologies reported appeared similar to those described by Krzanowska (1974), however the tail deformities observed in this study were not evident in Krzanowska's (1974) work. Nestor and Handel (1986) also reported reduced levels of morphologically abnormal sperm in the oviduct compared to the uterine population and suggested either active selection by the uterotubal junction against those sperm or some inherent physiological disadvantage reduced their number.

Experimental Considerations

In order to study the transport of morphologically abnormal sperm in the female reproductive tract, it is apparent that a number of factors must be taken into account before proceeding. The literature indicates that, from the sperm's perspective, transport is dependant upon the interaction of morphology and viability. To adequately study the process, reliable methods of sperm quality evaluation must be employed. Such methods would allow coincident determination of sperm morphology and some measure of sperm viability (motility

or mortality) of both the inseminated sperm and those recovered from the female reproductive tract.

Investigation of sperm interactions with the female reproductive tract present the researcher with unique problems. Quantitative and qualitative evaluation of sperm populations in the female tract involve removal of the sperm from the tract (see Van Demark and Moeller, 1950; Van Demark and Hays, 1955; Mattner, 1963a, 1966, 1968; Mattner and Braden, 1969a,b; Quinlivan and Robinson, 1969; Lightfoot and Salamon, 1970; Allison and Robinson, 1972; Thibault et al., 1975; Mitchell et al., 1985). Upon removal from the experimental animal, the tract has historically been flushed with physiological saline (Mattner, 1963a, 1966; Mattner and Braden, 1963; Lightfoot and Salamon, 1970; Allison and Robinson, 1970), 2.9% sodium citrate (Mitchell et al., 1985) or a weak hypochloride solution (Mattner and Braden, 1969b) after clamping, cutting or ligating the tract into the appropriate segments for study. The recovered flush fluid has been either centrifuged and a sample of the pellet counted using a hemocytometer (Lineweaver et al., 1970; Mitchell et al., 1985) or the flush fluid sampled without centrifugation and counted using a hemocytometer (Mattner and Braden, 1963; Mattner, 1966; Hawk et al., 1981) or counting wells (El Banna and Hafez, 1970; Hawk and Conley, 1975). Another method of sampling reproductive tract contents has been aspiration of the contents of a given region after partitioning the tract (Van Demark and Hays, 1955; Dobrowolski and Hafez, 1970).

Although considerable information has been gained using the techniques briefly described, a number of factors must be recognized in order to interpret the results. Because complete recovery of sperm from the female tract cannot be claimed, it must be assumed that the recovered sperm population is quantitatively and qualitatively a representative sample. Thibault et al., (1975) observed increasing numbers of sperm in the bovine uterine glands to 18 hours post-insemination. It would seem that the recovery of those sperm would be difficult and they could represent a distinct population compared to the uterine luminal population from a viability and morphology standpoint.

While sperm morphology, especially head shape, would not change in the time required to remove and flush the tract and concentrate the flush contents by centrifugation, changes in sperm motility or viability would undoubtedly occur during recovery and processing. Thus any viability or acrosome evaluation would be suspect. The use of a fixative to flush the excised tract would minimize degenerative changes in sperm quality traits during flush processing. Morphology of sperm could be preserved (Pursel and Johnson, 1974; Johnson et al., 1976), status of acrosomal integrity retained (Saacke and Marshall, 1968) and vital staining characteristics unchanged (Yip and Auersperg, 1972; Dott and Foster, 1975) by fixation thus, sperm morphology and viability could be assessed without time pressure.

Cross-contamination of regional sperm populations due to manipulation of the tract during removal at slaughter and the effects of gravity must be considered as potential sources of error in sperm population counts. Lineweaver et al., (1970) noted cervical mucus strands extending into the uterine cornua from the cervical ora of excised bovine reproductive tracts. Although this may have resulted from tract motility, it also may have been artifact introduced by tract manipulation at time of slaughter. Regional cross-contamination of the tract could be minimized by surgical removal of the tract prior to slaughter or excision of the tract after stunning but prior to exsanguination with the animal in dorsal recumbency. Ligatures and/or clamps would be placed immediately upon exposure of the tract *in situ*. Ligation or clamping of the tract to isolate the various regions would occur immediately upon its exposure *in situ*.

Due to animal sacrifice, measurement of within animal variation and repeated use of experimental animals is precluded. Large scale studies of sperm transport in animals larger than sheep are almost nonexistent undoubtedly due in large part to economic considerations. These factors, plus the importance of understanding the interactions of sperm with the female genital tract in such an economically important species as the bovine underscores the necessity of conducting well designed, adequately researched and closely controlled experimentation. Without such an experimental approach redundant results, or those having little new meaning would follow the sacrifice of costly experimental animals and expenditure of valuable time.

Experimental Rationale

In the dairy industry, and to a lesser extent in the beef cattle industry, a high proportion of cows are inseminated artificially, thus bypassing the naturally imposed barrier the cervix represents. As presented in the literature, the transport of sperm in the female reproductive tract appears to be a sperm morphology- and sperm viability-/motility dependant process that can be influenced by the bovine cervix. The functions of the bovine cervix may be i) a reservoir from which sperm migrate to effect fertilization and ii) a haven for sperm from polymorphonuclear leukocytic phagocytosis. Recent circumstantial evidence indicates that the cervix may act as a selector that filters out morphologically abnormal sperm. Bypassing the cervix with artificial insemination (A.I.) may eliminate a mechanism employed by the female for reducing the number of abnormal sperm reaching the site of fertilization, thus influencing the conception rate of certain bulls when used as A.I. sires. It is not known if the proportion of abnormal sperm in the uterus after vaginal semen deposition and subsequent cervical passage differs from that in the inseminate.

Recovery of inseminated sperm from the excised female tract for comparison to the inseminate has previously been used to study sperm interactions with the female genital tract. Vaginal insemination of cows with semen from bulls known to produce a high level of sperm having abnormal heads and comparison of the recovered uterine sperm to the inseminate would be direct evidence of sperm morphology types normally excluded from the female tract in natural service and would resolve the role of the cervix in this capacity. Such information would indicate whether the effects of cervical bypass with A.I. could contribute to reduced fertility in some bulls and, at the same time, would improve semen evaluation of potential A.I. sires of the future by indicating the type of sperm preferred by the female tract. Studies of cervical function, such as the research described herein, can only augment our understanding of the interaction of sperm with the female genital tract and clarify the role of sperm abnormalities in fertility.

Experimental Procedure

Introduction

The purpose of this investigation was to evaluate the ability of the bovine cervix to regulate the quality of sperm entering the uterus after vaginal semen deposition. Specifically investigated were differences in viability and morphology between the inseminated sperm and those recovered from the uterus after vaginal deposition and subsequent cervical passage. To address this question, two studies were conducted. Study #1, the Preliminary Study, was conducted to ascertain whether such difference occurred and, if so, to identify an appropriate time post-insemination to recover an adequate number of sperm for qualitative evaluation. In addition, methods for the recovery and evaluation of sperm viability and morphology were developed. This study will be referred to as Study 1 and was composed of three trials. Trial 1 and Trial 2 dealt with validation of vital staining techniques and Trial 3 with sperm transport. The second study was conducted to investigate the basis for the changes in sperm quality observed to occur in Study 1. This study will be referred to as Study 2 and its constituent experiments as Experiment 1 and Experiment 2.

Study 1 (Preliminary Study)

Validation of Vital Staining of Fixed Sperm.

Physiological fluids have historically been used to flush sperm from the female tract in sperm transport studies (see Experimental Considerations). In the present study, it was feared that erronious sperm viability results would occur, due to sperm ageing and death, if a physiological fluid was used. This was because of the time required to flush the tract, concentrate the recovered material and evaluate the sperm it contained. Thus, fixative was used as the flush fluid. Fixation procedures have been shown not to change sperm morphology (Pursel and Johnson, 1974; Johnson et al., 1976) or vital staining characteristics (Dott and Foster, 1975; Sekoni et al., 1981). Because sperm morphology and viability were to be evaluated, fixation of sperm appeared to be a solution to potential time-induced viability changes without jeapordizing the morphological evaluation. Although the morphological evaluation of aldehyde-fixed bull sperm has been validated (Johnson et al., 1976; Harasymowycz et al., 1976), the vital staining of aldehyde-fixed sperm using eosin-aniline blue has not.

Two trials were conducted to compare the vital staining of unfixed vs fixed sperm using semen collected by artificial vagina from mature Holstein bulls. Sperm concentrations were determined shortly after collection using a spectrophotometer as previously described. In all cases of fixation, sperm were added to freshly prepared glutaraldehyde-paraformaldehyde fixative (Karnovsky, 1965) at a semen to fixative ratio of 1:100 (v:v) and vigorously shaken for 15 seconds after combination. Preliminary work determined that this procedure reduced clumping of sperm and eliminated gelation of seminal plasma that occurred when higher proportions of semen were combined with fixative.

Smears of unfixed semen samples were prepared according to the procedure of Shaffer and Almquist (1948). Using a disposable 1μl serological loop, semen was mixed in a drop of room temperature stain that had been placed upon a microscope slide. A pair of smears were prepared by immediately placing another slide flat upon the semen-stain mix-

ture, allowing the mixture to spread between the two slides and then quickly sliding the slides apart in a horizontal fashion. Slides were rapidly dried using a hot air blower (45°C) then coverslipped with mounting medium. If more than 30 seconds elapsed between initial contact of semen to stain and the complete drying of the slide, the pair of smears were discarded and new ones prepared.

Smears of fixed sperm were prepared by placing a drop of fixed semen next to a drop of stain on a slide and tipping the slide so that semen and stain could merge. A disposable 1μl serological loop was used to completely mix stain and fixed semen and the mixture was smeared, dried and coverslipped as previously described. At least 15 minutes were allowed to elapse between placing semen in fixative and preparation of vitally stained smears of fixed semen.

All smears were coded and evaluated at 1250x magnification under oil with a brightfield microscope. Sperm were differentially counted with the number of unstained, stained (completely eosinophilic) and half-stained (only the posterior sperm head eosinophilic) recorded in a total of 200 randomly observed sperm in each smear.

Trial 1.

The effect of the method of killing bovine sperm on the results of vital staining of fixed and unfixed semen samples was investigated in this trial. Fresh undiluted semen, collected by artificial vagina from three different, mature Holstein bulls, was pooled such that each bull contributed equal numbers of sperm to the pool. The trial was repeated three times using semen from different bulls to form pools whose mean concentration was $9.4 \pm 1.4 \times 10^6$ sperm per ml. Three individual 2-ml samples from each pool were killed by either repeated freeze-thawing in liquid nitrogen, by heating to 62°C for 15 minutes in a waterbath or by incubation (ageing) at 37°C for 48 hours (Figure 1). Paired eosin-aniline blue vital stained smears were prepared from fixed and unfixed semen samples before and at the completion of each kill treatment. Smears were prepared and differentially counted as previously described, and the

results of vital staining of fixed samples were compared with those of unfixed samples both before and after kill treatment.

Trial 2.

This trial was conducted to compare the vital staining results for fixed and unfixed semen from samples to which known proportions of killed sperm had been added. Fresh undiluted semen, collected by artificial vagina from 3-4 different mature Holstein bulls, was combined and thoroughly mixed, then half the volume of the pool was killed by heating to 62°C for 15 minutes in a waterbath. Killed sperm were pipetted into samples of unkilled semen from the pool in graded volumes such that the combined samples contained 1%, 10%, 20%, 40%, 60%, 80% and 100% killed sperm (Figure 2). The trial was repeated twice using pooled semen from 3 and 4 different bulls. Sperm concentration in the 2 pools, determined spectrophotometrically, averaged $9.7 \pm 1.2 \times 10^6$ sperm per ml, to which the constituent bulls contributed a total of $24.9 \pm .3 \times 10^8$ and $29.4 \pm 4.3 \times 10^8$ sperm each to their respective pools. Eosin-aniline blue stained smears were prepared from fixed and unfixed samples of semen containing each level of added killed sperm. Vitally stained smears were prepared and differentially counted as previously described, and the results of vital staining of fixed samples were compared to those of unfixed samples for each level of added killed sperm.

Trial 3

Twelve nonlactating, uniparous Holstein cows were employed in this trial to determine whether the viability and morphology of the uterine sperm population after vaginal semen deposition was qualitatively different from that of the inseminate.

After calving, at least two normal estrous cycles were observed before utilization of any cow in the study. During that period, KAMAR® heat detectors were affixed to the cows, who were observed twice daily for behavioral signs of estrus. After day 10 of the second cycle post-calving, ovarian palpation per rectum was performed to determine presence of the corpus luteum, each cow was injected with a 25mg intramuscular dose of prostaglandin $F_{2\alpha}$ to

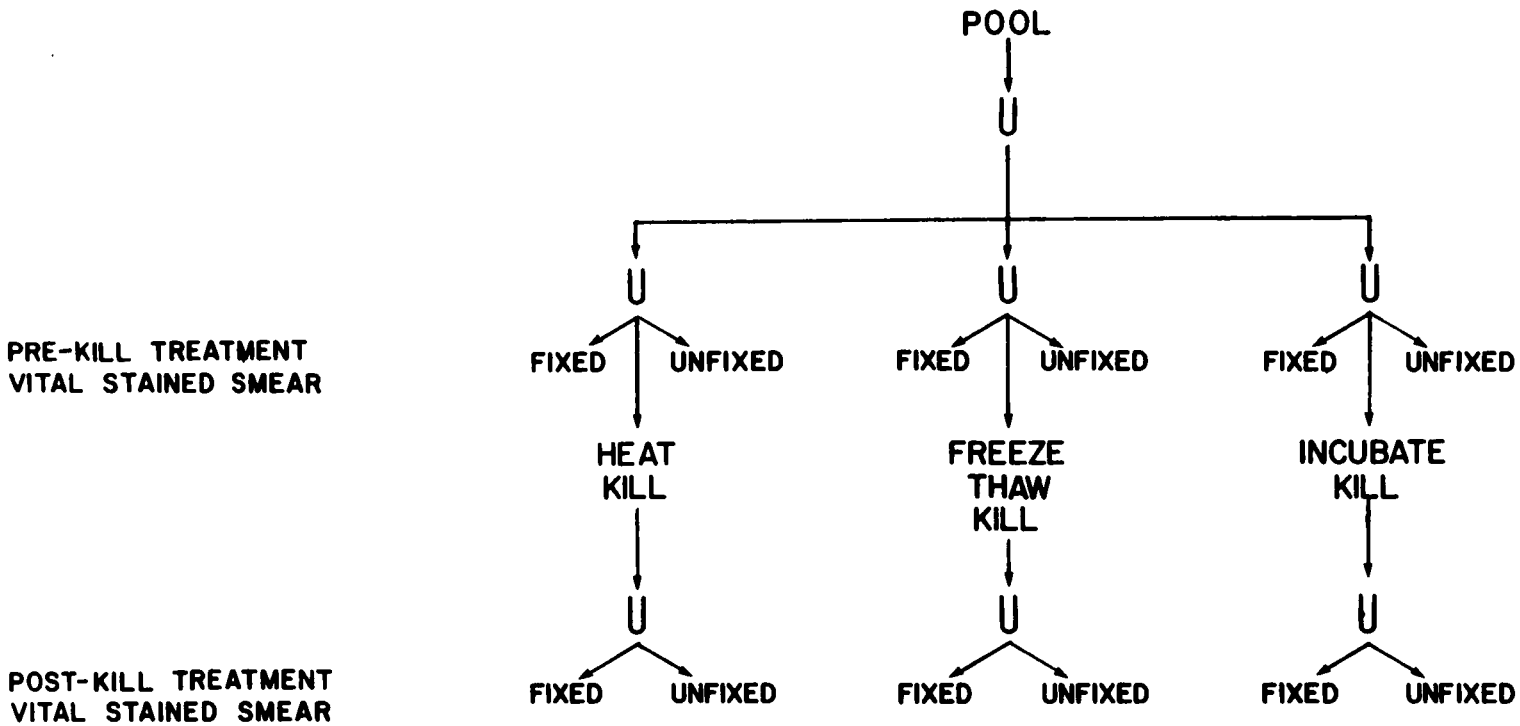

Figure 1. Experimental flow chart for trial 1. Sperm were killed by 3 methods to compare vital staining results for unfixed and fixed sperm before and after kill treatment.

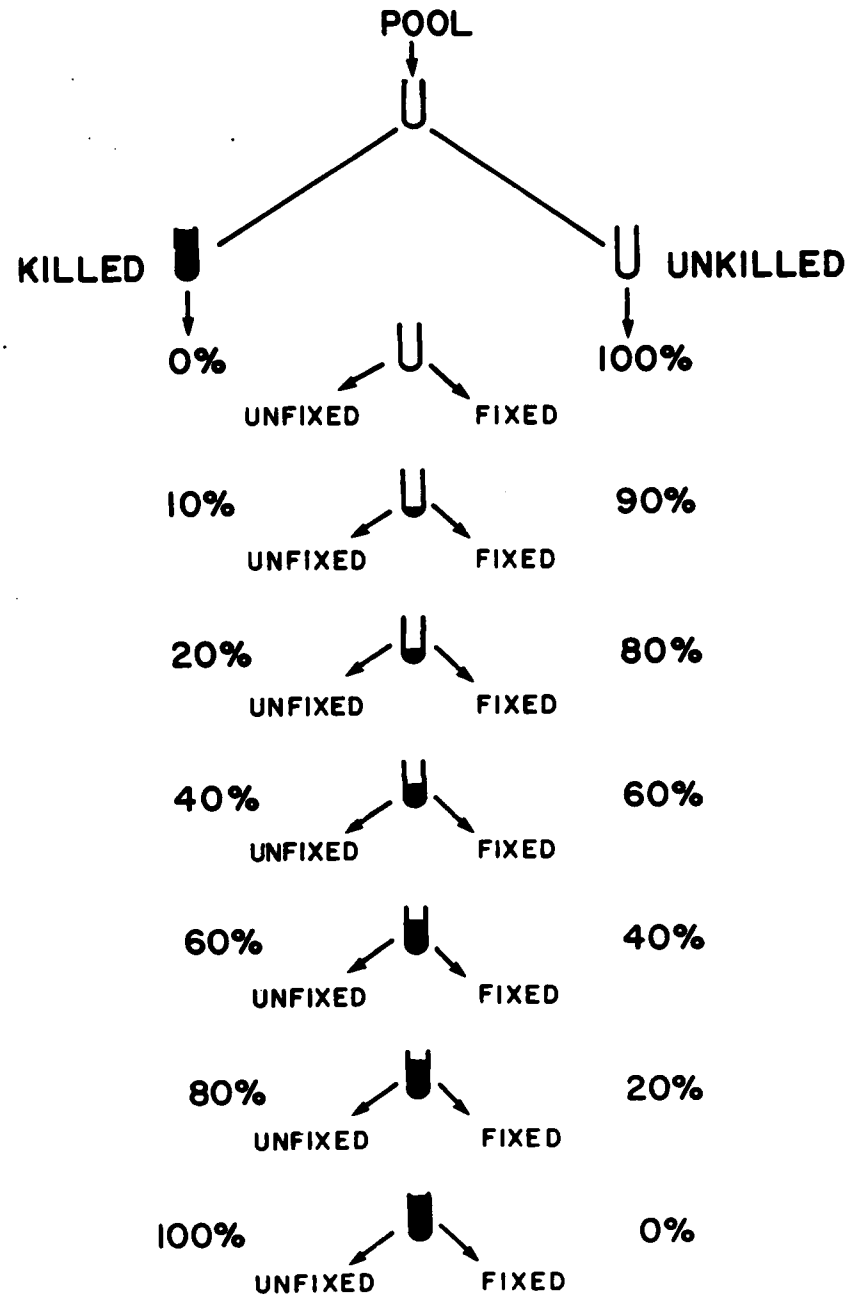

Figure 2. Experimental flow chart for trial 2. Vital staining results for unfixed and fixed sperm were compared after addition of graded proportions (v:v) of killed sperm to unkilled sperm.

induce estrus. Eighty hours after prostaglandin $F_{2\alpha}$ treatment (Lutalyse®) each cow was inseminated and randomly assigned to be sacrificed 4-, 8-, or 12 hours later to recover sperm from the reproductive tract. Experimental flow chart is presented in Figure 3.

Semen Preparation and Insemination

Each cow was inseminated with the unextended, pooled semen from two different bulls known to produce high levels of morphologically abnormal sperm. Two ejaculates were collected by artificial vagina from each bull and ejaculate volume, sperm concentration and motility were determined. Motility was subjectively estimated at 250x magnification using a phase contrast microscope whose stage temperature was maintained at 37°C using an air curtain (Sage Instrument Co.). Sperm concentrations were determined using a previously calibrated Bausch and Lomb Spectronic 20 spectrophotometer adjusted to 550 nm. Percent transmittance was converted to sperm numbers per ml using a conversion table validated by hemocytometer counts of sperm suspensions representing the range of percent transmittance given by the spectrophotometer. Ejaculate volume was determined by observing the semen level in relation to the volume graduations imprinted on the side of each collection vial.

One of the two ejaculates from each of the 2 bulls was chosen for pooling. The ejaculate having the greatest volume and percent motile sperm and whose sperm concentration exceeded 10^9 sperm per ml was chosen for use. The remaining ejaculate from each bull was centrifuged for 120 minutes at 5000 g after which the supernatant (seminal plasma) from each was decanted and used to dilute the respective bulls' chosen ejaculates to $1x10^9$ sperm per ml. The volume of the bull's seminal plasma necessary to dilute the chosen ejaculate was determined using the formula $V_iC_i = V_fC_f$ where V_i and C_i represent initial volume and concentration, respectively, and V_f and C_f represent final volume and concentration, respectively. After equal volumes of each adjusted ejaculate were pooled and thoroughly mixed, the sperm concentration and motility were again determined and the semen used to inseminate the designated cow.

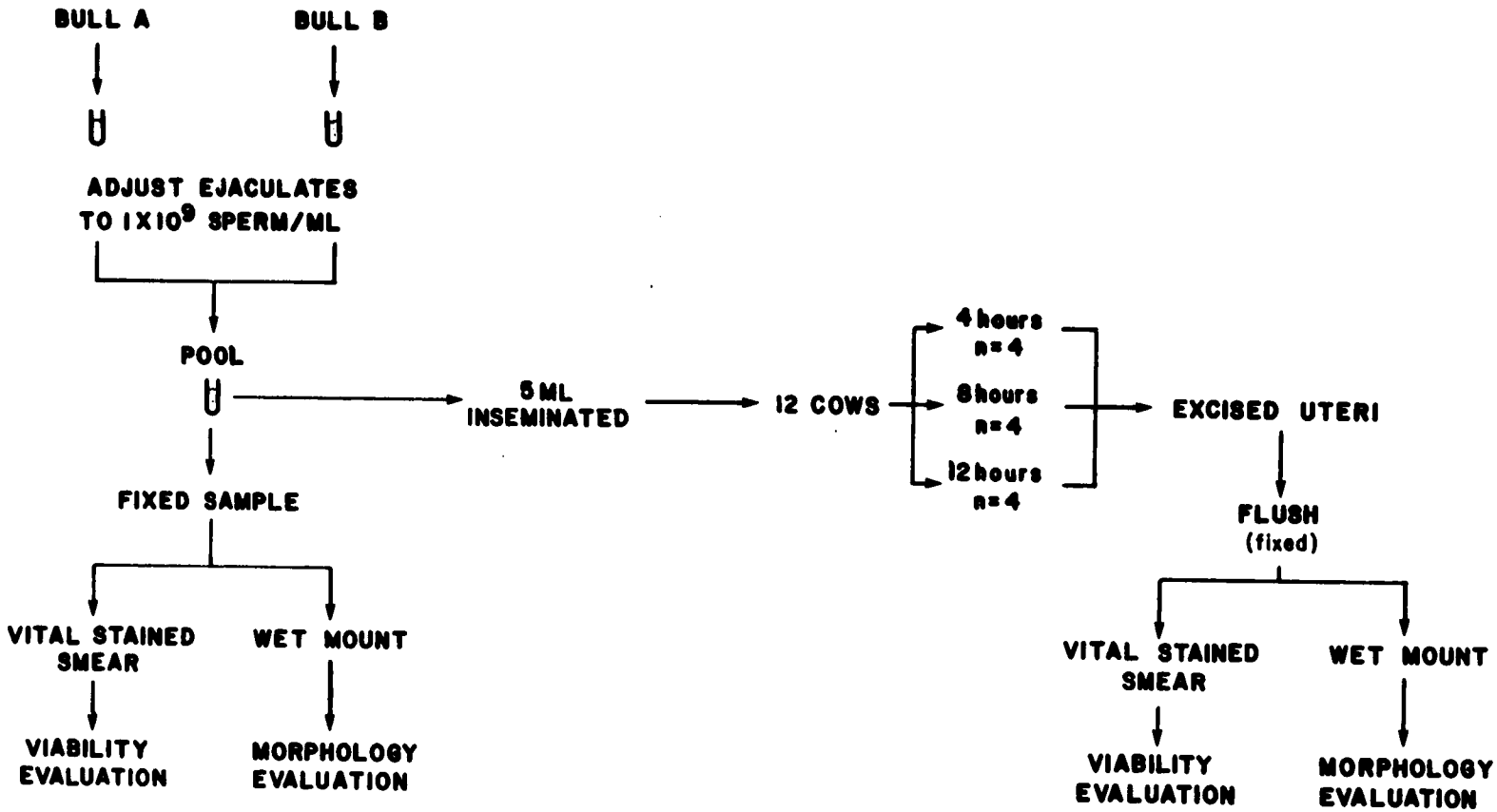

Figure 3. Experimental flow chart for preliminary sperm transport trial. Viability and morphology of vaginally-inseminated sperm were compared to that recovered fro the uteri of 12 cows sacrificed 4-, 8-, or 12 hours after insemination.

Five ml pooled semen was deposited in a circular fashion at and around the external cervical os using a plastic inseminating rod connected to a 10ml syringe. As the syringe plunger was depressed to deposit the semen, the inseminating rod was rotated about the face of the external os. The inseminate volume, sperm concentration and manner of semen deposition were employed to duplicate as closely as possible the quantity and distribution of semen that occurs with natural service. A sample of the inseminate was preserved for later evaluation by combining 100 μl semen with 1ml glutaraldehyde-paraformaldehyde fixative (Karnovsky, 1965) in a 1.5 ml plastic vial labeled with the cow's number and the origin of the contents (i.e. inseminate).

Slaughter and Track Removal

Each cow was sacrificed 4-, 8- or 12 hours post insemination. After stunning with a captive bolt device and exsanguination, the reproductive tract was excised with the cow in dorsal recumbency. Prior to tract removal, cord ligatures were placed at the internal and external cervical ora as determined by careful palpation of the tract *in situ* upon its exposure following incision of the abdominal wall. The uterotubal junctions and oviductal ostia were clamped with hemostats to prevent further loss or relocalization of tract contents during tract removal and subsequent transport to the laboratory. Upon excision, each tract was placed on a room-temperature plastic foam tray, then enclosed in a plastic bag for transport to the laboratory.

Sperm Recovery

Upon arrival at the laboratory, the clamped and ligated tract was cut transversely as close to the internal cervical os as could be determined by palpation, thus separating the uterus from the cervix, vagina and vulva. An 11 inch curved Rochester Pean forcep was immediately placed as closely as practical to the cut end of the uterus to clamp it closed. The tract was then suspended, cornua down, by securing the forceps in a right-angle screw clamp attached to a ring stand holder (Figure 4). The mesometrium was then quickly trimmed away

to allow the uterine cornua to uncurl, and two short, longitudinal incisions were made as caudally as possible through the dorsal uterine wall, one left- and one right lateral to the uterine midline, to access the uterine lumen. The incisions were located to correspond with the lumen of each uterine cornu in the event the internal uterine bifurcation extended to a point such that both cornual lumens were not accessible via one incision.

Glutaraldehyde-paraformaldehyde fixative (Karnovsky, 1965) was injected through each incision until the uterus was filled to the level of the incisions (≃250 ml) then the incisions were closed with hemostats (Figure 4). The uterus, which at this point exhibited some considerable distension, was then repeatedly manipulated so as to rinse the entire endometrial surface with fixative. After more than 15 minutes had elapsed, uteri were drained of fixative, endometrium was exposed by making a longitudinal incision from the most cranial to the most caudal aspect of each cornu. The exposed endometrium was then reflushed with an additional 50 ml fixative per cornu delivered by syringe and the two flushes combined. The uterine flushings were sequentially centrifuged at 2000 g for 15 minutes to reduce volume and to concentrate recovered material, which was then placed in 1.5ml plastic vials labeled with the cow's number and the origin of the contents (i.e. uterine flush).

Sperm Morphology and Viability Evaluation.

Vials containing the fixed inseminate samples and the uterine flush material were coded and sperm viability and morphology were assessed by counting 200 sperm from a sample of each. Sperm morphology was evaluated using wet mounts prepared from inseminate and uterine flush samples that were viewed under oil at 1250x magnification with differential interference contrast optics. Specific abnormalities of the sperm head, tail and presence and location of protoplasmic droplets for each abnormal sperm were recorded based on the types reported by Lagerlöff (1934) and Blom (1973). Wet mounts were prepared by placing a small drop of sample on a microscope slide then coverslipping with gentle pressure. Sperm viability was separately determined using vitally stained smears prepared from fixed samples of the inseminates and uterine flushes inspected at 1250x magnification

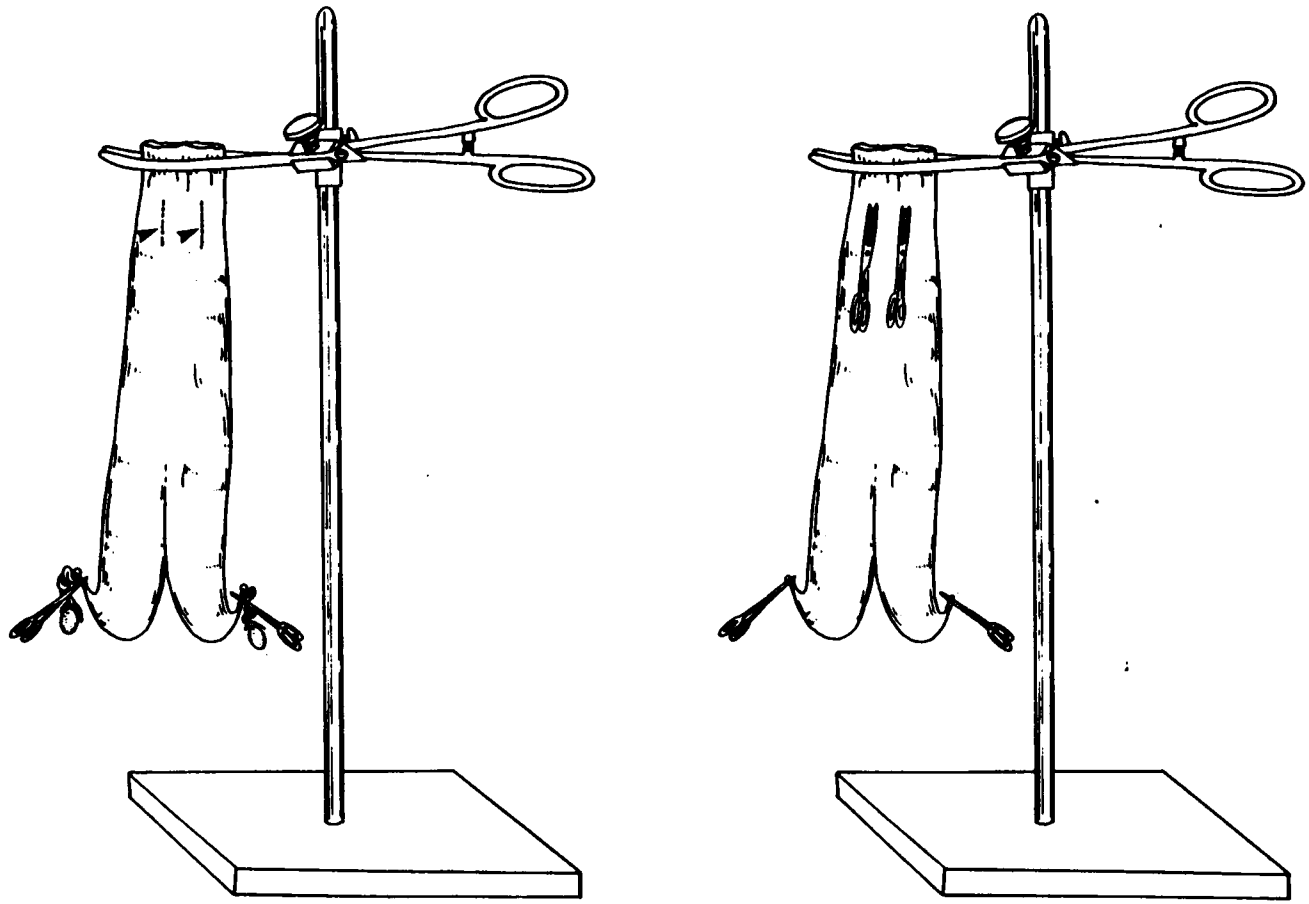

Figure 4. Excised uteri suspended from forceps secured to a ring stand holders. Left: Location of incisions through which fixative was introduced into the uterine lumen is indicated by arrows. Right: Incisions closed by hemostats after addition of fixative.

under oil with bright-field microscopy. The procedure of differential vital staining using eosin-aniline blue (Shaffer and Almquist, 1948) as described by Saacke (1970) was modified for use on fixed sperm. Validation procedures of this modification for semen preserved in glutaraldehyde-paraformaldehyde fixative (Karnovsky, 1965) are presented in Trial 1 and Trial 2 of this study under the heading Validation of Vital Staining of Fixed Sperm.

Study 2

Two experiments were conducted to investigate the basis for the differences in sperm quality that resulted between the inseminate and uterine sperm populations after vaginal semen deposition in Study 1. A cervical effect was suggested by the preliminary results because differences occurred between the inseminate and uterine sperm populations after vaginal semen deposition; however, a uterine effect could have been just as likely a cause for the differences because the uterus was the location from which sperm were recovered. To separate uterine from cervical effects on the quality of the uterine sperm, pairs of estrus heifers were inseminated in each experiment such that intrauterine deposition (no cervical passage of sperm prior to entering the uterus) in one heifer of each pair served as the control for the effects of vaginal deposition (cervical passage of sperm prior to entering the uterus) in the other heifer of the pair.

To define the nature of the effect the cervix had on the quality of sperm passing through it (if such an effect did exist), samples of cervical contents were taken from the central and more peripheral aspects of the cervical canal as well as each quarter of the cervix in a cranial-caudal direction. The sampling of cervical quarters was expected to indicate any progressive changes in sperm quality that occurred as sperm moved towards the uterus after vaginal semen deposition. After intrauterine insemination, such sampling was intended to serve as a control for any effects of vaginal deposition. Contents of the central and more peripheral aspects of the cervical canal in each cervical quarter were sampled to determine

sperm quality differences between the two locations. The literature indicates that sperm in the deeper aspects of the cervical mucosa exhibit a higher viability than those found in the more central aspects of the cervical canal (Mattner, 1966; Mattner and Braden, 1969a; Lightfoot and Restall, 1971) and are believed to be those sperm involved in sustained transport through the cervix to the uterus (Mullins and Saacke, 1989). It is not known, however, if sperm viability differences between the central and peripheral aspects of the cervical canal are accompanied by differences in the morphology of the sperm.

In order to separate sperm viability-related effects from the effects of sperm morphology on the transport of sperm in the female and to avoid confounding of results, the viability and morphology must be evaluated for each sperm counted. For this reason, the coincident viability and morphology of each sperm was determined in Experiment 1 and Experiment 2. To further separate the effects of sperm viability from those of sperm morphology, the proportion of live sperm in the semen used in Experiment 1 was intentionally much greater than that used in Experiment 2 (i.e. 91% vs 32% intact acrosomes, respectively). It was reasoned that viability effects on changes in sperm morphological quality between the inseminate and uterus would be least pronounced when high viability semen was used, thus allowing any morphology effect *per se* the best opportunity to become evident. Conversely, the use of low viability semen in Experiment 2 was anticipated to cause pronounced sperm viability effects and result in reduced or undetectable sperm morphology effects on differences in sperm quality between the inseminate and uterine sperm populations.

In the event that sperm filtration by the cervix became evident based upon the morphology of sperm passing through it, a test of the organ's ability to differentiate gross from subtle sperm head abnormalities was devised. Figure 5 depicts examples of sperm head abnormalities considered to be gross and subtle, illustrating the range in severity of head abnormalities encountered in this study. In Experiment 1, when morphology effects (if present) were expected to be most pronounced, the evaluation of sperm head morphology was stringent, which included the subtle abnormalities with the gross forms when abnormals were counted. In Experiment 2, when morphology effects were expected to be least pronounced

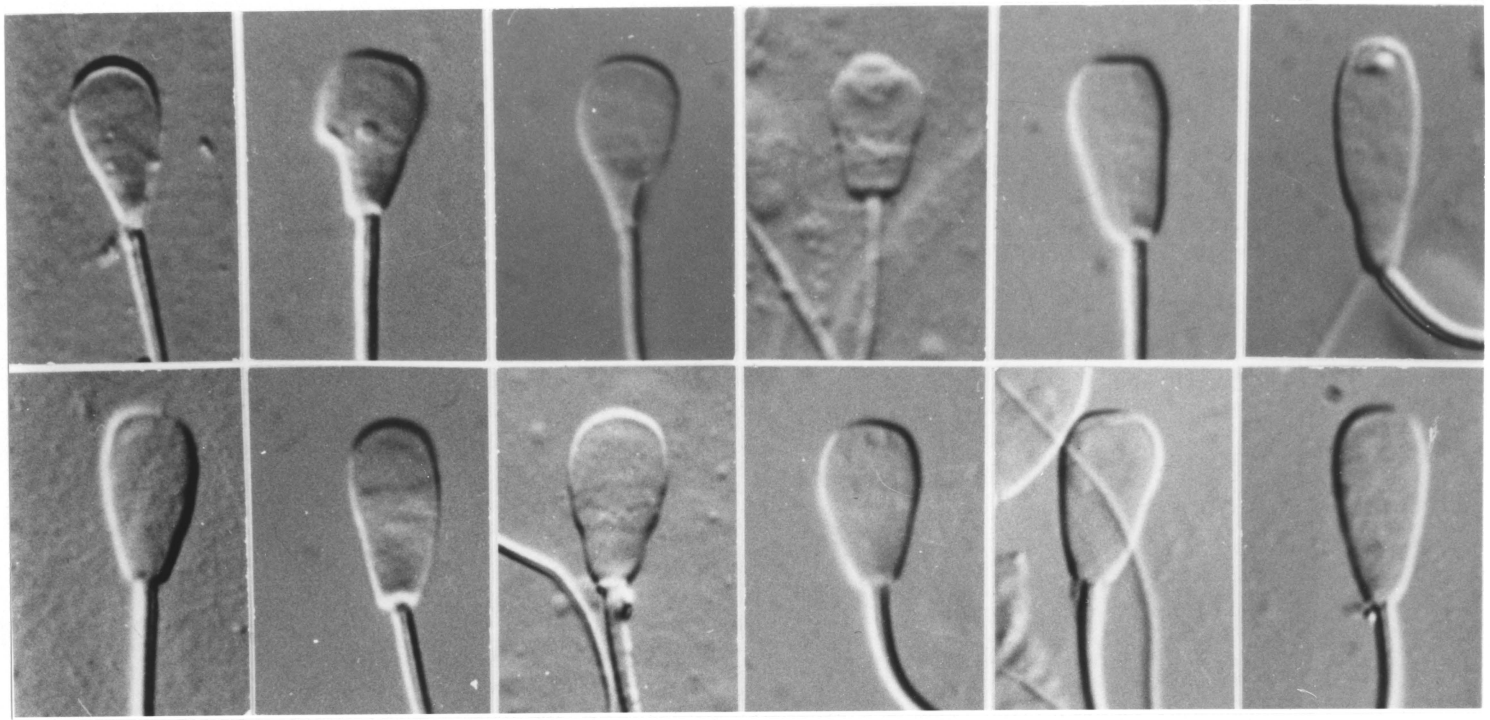

Figure 5. Sperm head abnormalities and the range in their severity encountered in Experiment 1 and Experiment 2. Left to right: Tapered, asymmetrical, pyriform, short, flat, elongated. Top row: Gross abnormalities. Bottom row: subtle abnormalities.

due to the use of low-viability semen, sperm morphology was less stringently evaluated and only the grossly abnormal sperm were counted as abnormal; the remainder were counted as having normal head morphology.

Experimental Animals

This study involved the use of 8 pairs of virgin Holstein heifers to pursue in more detail the specific objectives outlined in the introduction, particularly objectives b, c and d. The heifers were originally potential replacement females for the VPI&SU Dairy Center milking herd but had been culled based upon non-reproductive criteria.

At the beginning of the study, the reproductive tracts and ovaries of all heifers were examined by rectal palpation to eliminate from the experimental animals any individuals with perceptable anomalities of the tract or ovaries. All heifers were affixed with KAMAR® heat detectors and those with functional corpora lutea were injected with a 25 mg intramuscular dose of prostaglandin $F_{2\alpha}$ to synchronize estrus. Heifers were observed 2 to 3 times daily for behavioral signs of estrus which were recorded throughout the study.

When a pair of heifers exhibited behavioral estrus simultaneously, they were removed from the rest of the heifers and the presence of a preovulatory follicle was quickly determined by gentle ovarian palpation per rectum. Low blood progesterone was confirmed using an enzyme linked immunosorbent assay (ELISA) of blood obtained by jugular venipuncture. Only pairs of heifers exhibiting estrus behavior and having ELISA and ovarian palpation results indicating a preovulatory condition were used. After confirmation of estrus, heifers were inseminated and sacrificed 12 hours post-insemination to recover sperm from their reproductive tracts.

Semen Preparation and Insemination.

Each pair of heifers was inseminated with fresh, unextended semen from single bulls, or semen pooled from 2-4 bulls, all known to produce relatively high levels of morphologically abnormal sperm. Bulls were specifically chosen for use if their sperm exhibited high pro-

portions of abnormal head morphology, retained normal flagellar morphology and their motility indicated a high degree of viability. Semen meeting these criteria was used to eliminate confounding of results due to impaired motility which has been proposed as a deterrent to the passage of sperm through the female tract (Mortimer, 1977; Mitchell et al., 1985). Methods of semen collection and inseminate preparation were identical to those in Study 1.

To separate uterine from cervical effects on the elimination of sperm having abnormal head morphology, semen was deposited in the vagina of one heifer of the estrus pair and in the uterus of the other. For vaginal semen deposition, 5 ml semen was deposited in a circular fashion at and around the external cervical os using a plastic inseminating rod connected to a 10 ml syringe. As the syringe plunger was depressed to deposit the semen, the inseminating rod was rotated about the face of the external os. For intrauterine deposition, .5 ml semen was drawn into the inseminating rod using the attached syringe, the rod was guided through the cervix and semen was deposited in the caudal uterine corpus. The volume of semen and number of sperm deposited in the uterus were chosen to duplicate the semen volume deposited under artificial insemination conditions and to approximate the degree of reduction in sperm numbers that appeared to occur after cervical passage of vaginally deposited sperm in cattle (Dobrowolski and Hafez, 1970). Upon deposition of semen, clitoral massage was performed for 5 seconds based upon the assumption that the resultant contractions of the genital tract (Cooper et al., 1985; Cooper and Foote, 1986) would mimic those that occur in response to natural mating (Van Demark and Hays, 1954).

An aliquot of the inseminate was preserved for glutaraldehyde-paraformaldehyde fixative (Karnovsky, 1965) for later morphological and viability assessment. Mortimer (1977) raised the question of differential survivability of morphologically normal vs abnormal sperm as a factor negatively affecting the retention of abnormal sperm in the genital tract of the doe rabbit. To test whether differential viability of normal vs. abnormal sperm occurred in the present experiment, a 400μl sample of the inseminate was incubated *in vitro* at 37°C in 4ml of an extender composed of 20% egg yolk in 2.9% sodium citrate (EYC) to which no antibiotics had been added. Semen was pipetted into prewarmed EYC and incubated in a dry bath until

heifers were sacrificed. All of the EYC used in the study was prepared in one batch at the study's outset and was stored at -1°C as 4ml aliquots in 5ml plastic tubes.

Retrograde mucus was collected in a separate plastic beaker for each heifer as it exited the vulva during the 6 hour period immediately following insemination to test differential removal of abnormal vs normal sperm in the mucus. This time period was chosen because Mitchell et al. (1985) reported recovery of most of the retrograde loss in the 6 hour period following insemination. Fixative was added to mucus immediately upon its collection at approximately a 1:1 (v:v) ratio. Greater proportions of fixative hydrated the mucus, causing extreme liquification and therefore difficulties in handling and processing.

Slaughter and Tract Removal

Each pair of heifers was sacrificed 12 hours post-insemination. Slaughter and tract removal procedures were identical to those in the Study 1. Care was taken to prevent loss of vaginal mucus from the vulva. Urine contamination of vaginal contents was prevented by draining the bladder through an incision after removal of the tract and before transport to the laboratory.

Sperm Recovery

Upon transport of the excised genital tracts to the laboratory, a 200μl sample of the EYC-incubated inseminate was placed in 1ml fixative. Thus, the *in vitro* incubation period of sperm was brought into closest approximation to their *in vivo* incubation period prior to recovery from the tract. The uteri were separated from the more posterior portions of the tract and flushed in a manner identical to that used in the Study 1. The uterine lumen was filled with glutaraldehyde-paraformaldehyde fixative injected through two small incisions made in the caudal aspect of the dorsal uterine wall after the cut end of the uterus was clamped with large forceps and suspended from a ring stand holder. Fluid was drained from the uterus into a beaker, the dorsal wall of each cornu was incised along its entire length, the endometrium

was reflushed with additional fixative followed by phosphate buffer, which was pooled with the flush fluid previously collected from that trace.

A 3 ml aliquot of the mucus that remained in the anterior vagina was collected by aspiration using a syringe and then expressed into a vial containing a similar volume of fixative. These mucus samples were all collected to test differential removal of abnormal vs normal sperm in mucus.

To test for progressive changes in sperm quality along the length of the cervix, samples of its contents were taken from the central and peripheral aspects of the cervical canal in each quarter of the organ. Cervices were separated from the vaginae and their serosal layer dissected away. They were then opened by a single longitudinal incision through the dorsal wall, pinned down so as to expose the mucosa and cut transversely into quarters (Figure 6). A sample of the sperm population in the cervical lumen mucus was recovered from each cervical quarter by gentle curettage of the exposed epithelium using microscope slides (Figure 6). Each curettage sample was obtained using a clean, unused microscope slide which was discarded after use. Samples were placed in separate vials of fixative labeled with the animal's number and sample location. A vacuum device (Figure 6) was then used to sample the sperm population within the numerous large and small longitudinal folds of the cervical mucosa. Vacuum sampling took place with each of the cervical quarters in a small volume of fixative in separate labeled plastic weighing pans to prevent mucus dehydration and to preserve sperm. Capillary action tended to pull fixative between the folds, facilitating sperm preservation and recovery of the material between cervical folds. Small volumes of fixative were periodically aspirated through the vacuum device tip to rinse any adhering material from its internal walls. A clean vacuum device was employed for each cervical quarter and tips were discarded after use.

All recovered samples were concentrated by centrifugation (if necessary) as described in the Study 1 and each placed in 1.5 ml plastic vials labeled with the animal's number and origin of contents.

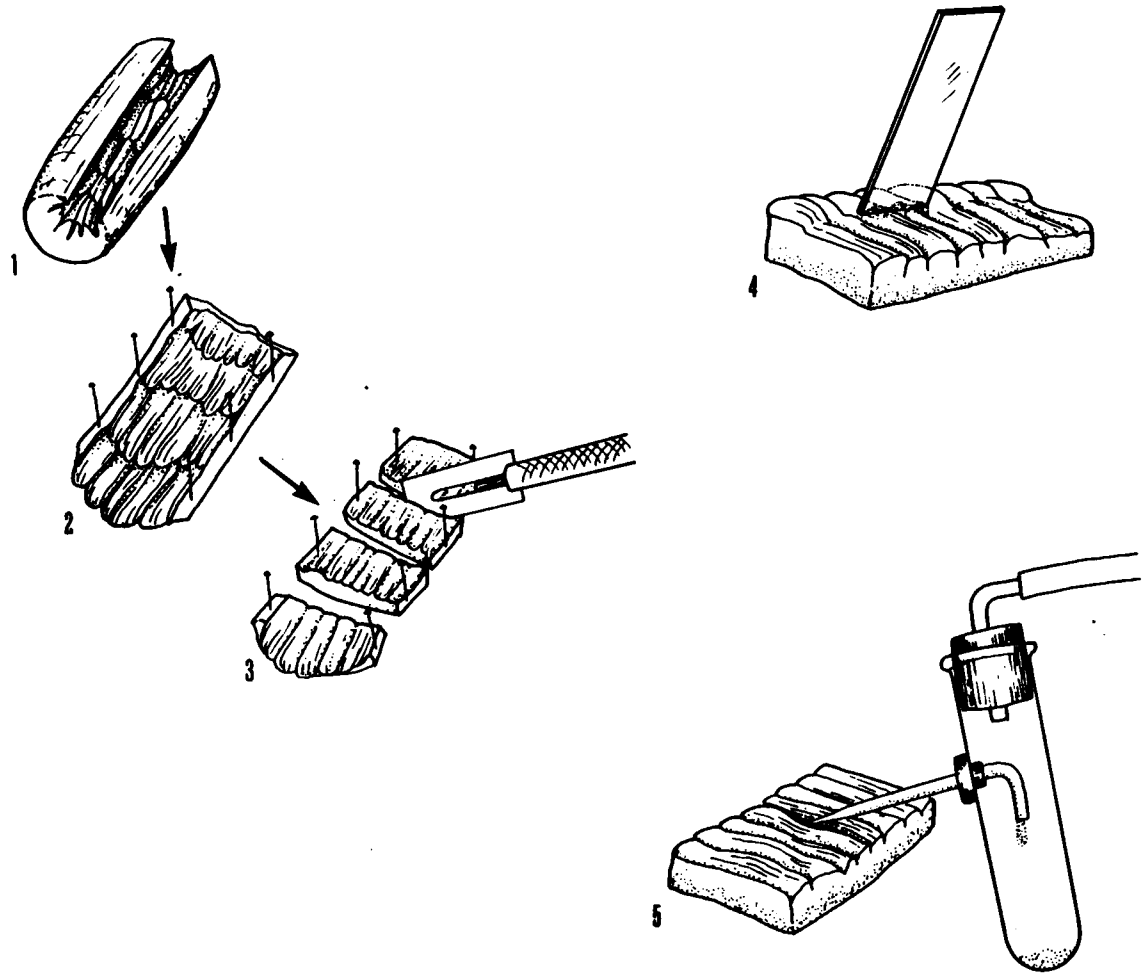

Figure 6. Method of obtaining samples from the central and peripheral cervical canal. 1-3: Dividing the cervix into quarters. 4: Curettage of mucosa in central cervical canal using a microscope slide. 5: Vacuum sampling of peripheral cervical canal between folds of cervical mucosa.

Immediately after fixative was drained from the uteri and the endometrium rinsed with fixative and phosphate buffer, two random samples of endometrium, approximately 2 cm square, were removed and placed in 10% formalin. These samples were taken simply as a check to determine efficiency of removal of uterine sperm by the flushing technique in light of Thibault's (1973) observation of sperm presence in the bovine uterine glands. The fixed endometrial tissue was embedded, sectioned, mounted on slides and stained to inspect for the presence of sperm in the uterine glands. Sections were viewed at 1250x magnification under oil using brightfield optics.

Sperm Morphology and Viability Evaluation

All recovered genital tract samples (retrograde mucus, vaginal mucus, vacuum and curettage cervical samples, uterine flush, inseminate and EYC incubate) were coded and evaluated for sperm viability and morphology. Wet mounts were prepared from each sample and were viewed at 1250x magnification under oil using differential interference contrast microscopy. Each of 200 sperm counted per sample was concurrently classified as to viability and morphology. Viability was assessed based upon acrosomal integrity (Saacke and Marshall, 1968) and sperm morphological abnormalities recorded as in the Study 1. Categorization of sperm abnormalities followed that of Saacke and White (1972), i.e., primary (head), secondary (protoplasmic droplet) and tertiary (tail). Designation of the abnormality type for sperm exhibiting multiple abnormalities of the head (e.g. tapered head with craters) was based upon the shape abnormality with a notation made of the presence of additional abnormalities. For sperm exhibiting abnormality combinations from different categories of abnormalities, (i.e. tapered head with distal droplet) the designation of the abnormal type was based upon primary abnormalities taking precidence over secondary abnormalities, which, in turn had precidence over tertiary abnormalities. A notation was made of the presence of multiple abnormalities.

Experiment 1

Five pairs of virgin Holstein heifers were inseminated with semen of high viability (i.e. 91% intact acrosomes) from bulls selected for their production of semen containing relatively high proportions of sperm having head abnormalities. High viability semen was used in this experiment to minimize any potential effects of sperm viability on sperm transport, thus allowing any morphology effect (selection based upon sperm morphology) the best opportunity to become evident. In three of the heifer pairs, each was inseminated with semen from single but different bulls, while the remaining two pairs were inseminated with pooled semen from 2 and 3 different bulls, respectively. Bulls contributing to semen pools were equally represented within their pools on a sperm numbers basis. The mean concentration of the inseminates was $1019.4 \pm 29.5 \times 10^6$ sperm per ml. Sperm head morphological evaluation in this experiment was stringent, in which subtle forms of head abnormalities (see Figure 5) were included with gross forms when abnormals were counted. The experimental flow chart for this experiment is shown in Figure 7.

Experiment 2

Semen of low viability (i.e. 32% intact acrosomes) was used in this experiment as a contrast for the high viability semen employed in Experiment 1. The use of this semen was expected to reveal pronounced sperm viability effects on sperm transport in the female, and thus permit evaluation of morphology effects under low viability conditions. Three pairs of virgin Holstein heifers were inseminated in the same manner as in Experiment 1. Semen containing high levels of sperm head abnormalities was collected from bulls induced to produce such sperm by artificial elevation of their scrotal temperature to $\simeq$ 38°C. Using sacs lined with insulating material and fitted with velcro closures, scrota were insulated for 24 to 48 hours and 9-14 days after removal of insulation, abundant sperm with head abnormalities were present in the semen. The initial effects of scrotal insulation were manifested by increased levels of protoplasmic droplets and aberrant tail morphologies, which became reduced with time as levels of sperm head abnormalities increased. Head abnormalities initially

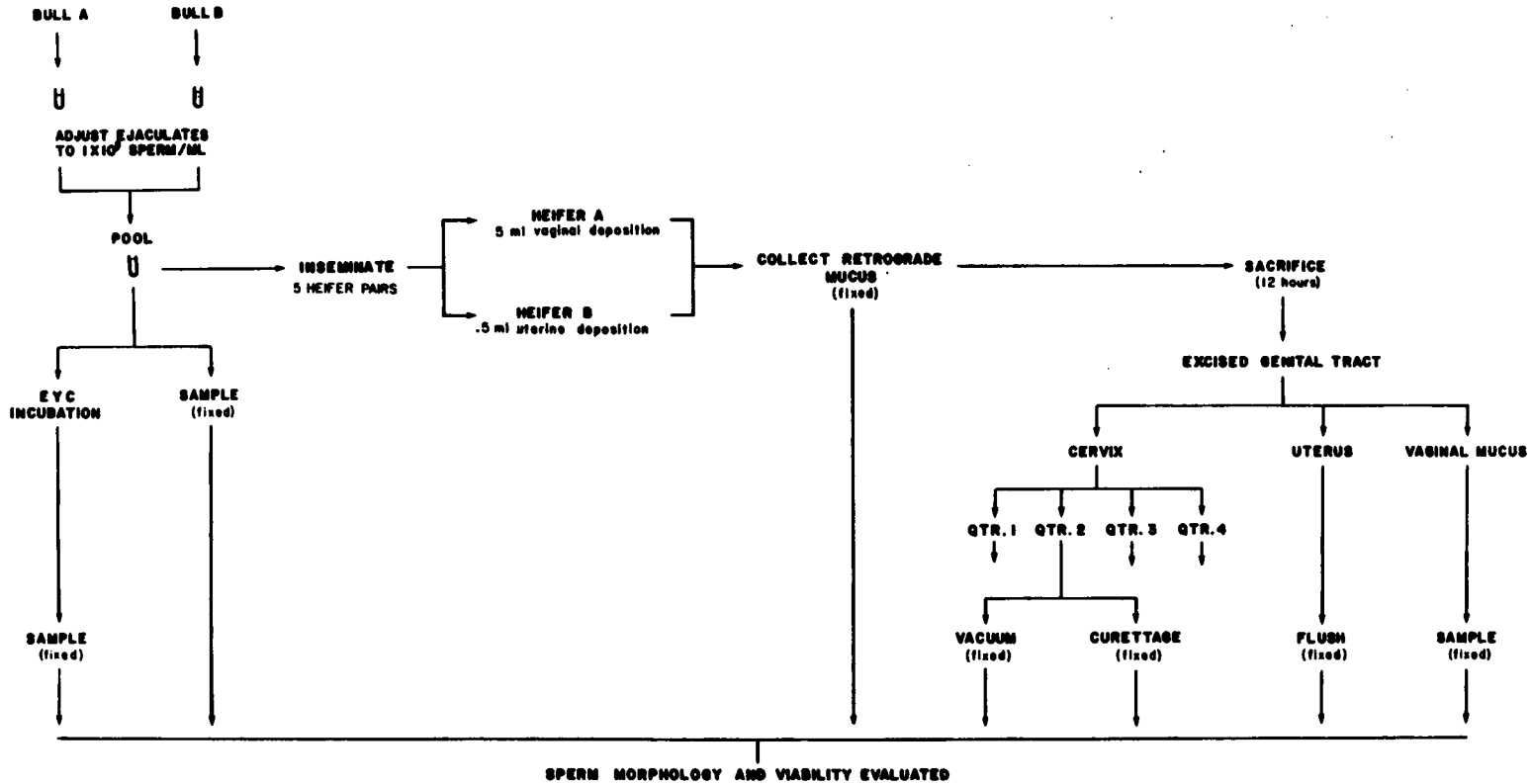

Figure 7. Experimental flow chart for Experiment 1. Five pairs of heifers were inseminated with high viability semen and the viability and morphology (coincidentally determined) of sperm inseminated and recovered from the tracts were compared.

consisted of craters and the diadem defect, which persisted to some degree as abarrant head shapes (particularly pyriforms, tapers and asymmetrics) became more pronounced. As the head shape abnormalities became the dominant type, midpiece and tail defects all but disappeared. Thus, semen containing high levels of motile sperm having head abnormalities could be obtained virtually on demand.

To test exclusion by the cervix of sperm based upon viability, half the volume of each inseminate was killed by repeated freeze-thawing in liquid nitrogen. To present numbers of viable sperm comparable to those inseminated in Experiment 1, yet maintain the inseminate volumes used in Experiment 1, the sperm concentration of the inseminate was intended to be $2x10^9$ sperm per ml, double that of Experiment 1. The intent, therefore, was two-fold; to continue testing the exclusion of sperm having abnormal heads and to test the exclusion of sperm by the cervix based upon viability.

The three heifer pairs were inseminated with pooled semen collected from 3, 3 and 4 different bulls, respectively. The constituent bulls did not contribute equal sperm numbers to their respective semen pools, as a result of the reduced semen volume and sperm concentration obtained from scrotally insulated bulls, and the inseminate sperm concentration requirement of $2x10^9$ sperm per ml. Mean concentration of the inseminates was $1946.3 \pm 85.5x10^6$ sperm per ml. Sperm morphology was not as stringently evaluated as in Experiment 1, with only the grossly abnormal sperm (Figure 5) counted as abnormal; the rest were counted as normal. The experimental flow chart for this experiment is shown in Figure 8.

Statistical Analysis

The data from this investigation was analyzed using the General Linear Models (GLM) procedure contained in the Statistical Analysis System (SAS; Spector et al., 1985). Means

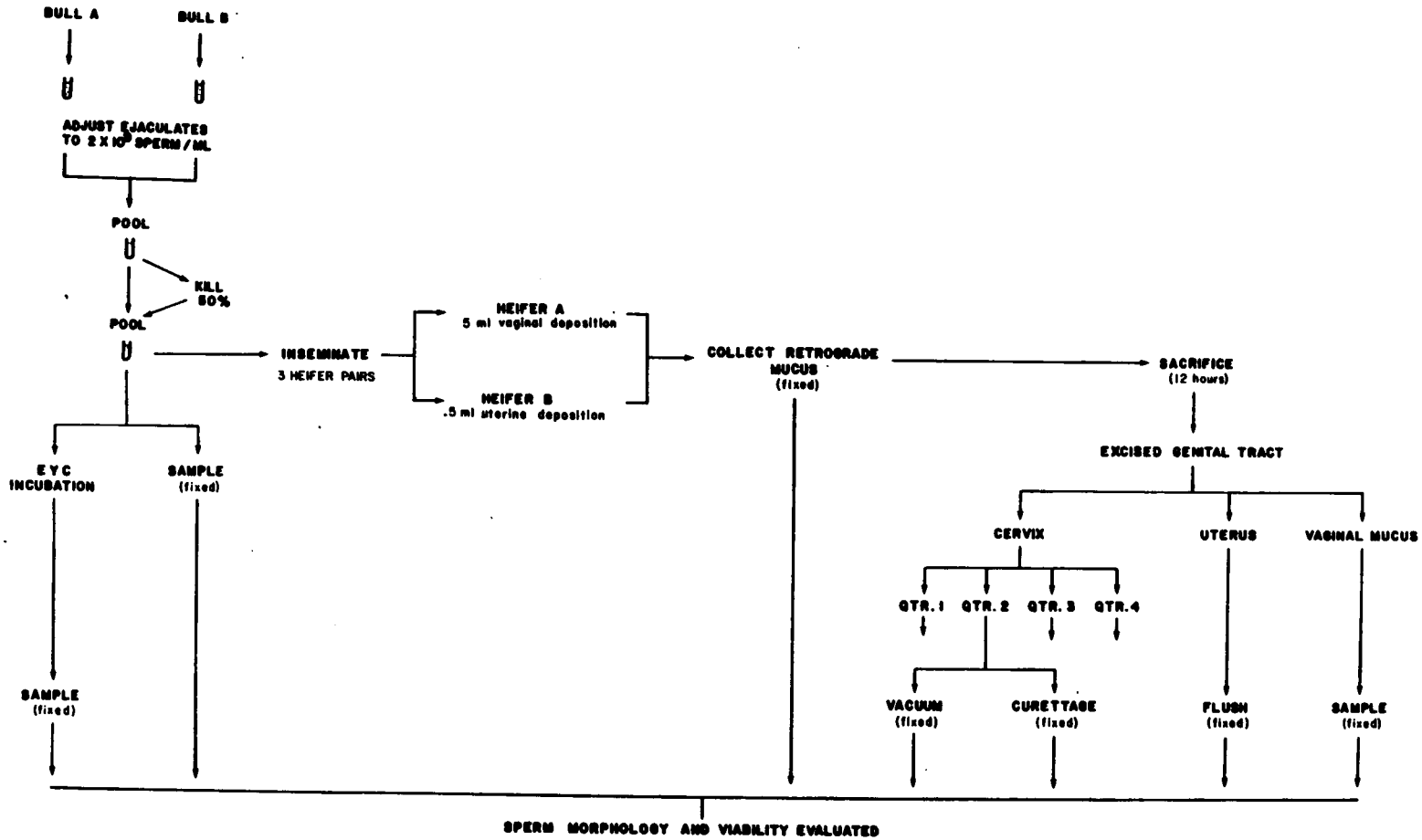

Figure 8. Experimental flow chart for Experiment 2. Three pairs of heifers were inseminated with low viability semen and the viability and morphology (coincidentally determined) of sperm inseminated and recovered from the tracts were compared.

comparisons, all of which were preplanned, were performed using the GLM 'contrast' specification. The Bonferroni t^2 was used to test significance in the event of nonorthogonality.

Study 1 (Preliminary Study)

The three data sets generated by this study were analyzed using four procedures. The data from Trial 1 were analyzed using the model:

$$Y_{ijk} = \mu + r_i + t_j + e_{ijk},$$

where

Y_{ijk} = the individual observation for a given variable,

μ = the overall mean,

r_i = the random effect of the i^{th} experimental repetition,

t_j = the fixed effect of the j^{th} kill method-fixation method-time combination, and

e_{ijk} = the error term.

Dependant variables % unstained, % half-stained and % unstained plus % half-stained represented the average for the pair of smears prepared from a sample of each kill method-fixation method-time subclass, where 200 sperm were randomly counted per smear. Data were transformed using the method $\sqrt{\text{observation}} + \sqrt{\text{observation}+1}$. Method of transformation was selected based upon criteria proposed by Kirk (1982). Because the effects of the interaction of kill method (heating, freeze-thawing, incubation), fixation method (fixed, unfixed) and time (pre-kill, post-kill) were of primary interest, each kill-fixation-time subclass was given a treatment designation, using the formula Treatment=(kill x 100)+(Fix x 10)+Time, then analyzed. Preplanned comparisons were performed between least squares means of each fixation method within each kill method-time subclass and among kill methods within each fixation method-time subclass (Appendix Table 1). Although data were transformed before analysis, least squares means of the untransformed data are reported but with significance based upon results of analysis of the transformed data.

The data from validation Trial 2 were analyzed using the model:

$$Y_{ijkl} = \mu + t_i + p_j + (tp)_{ij} + o_{k(ij)} + e_{ijkl},$$

where

Y_{ijkl} = the individual observation for % alive,

μ = the overall mean,

t_i = the fixed effect of the i^{th} fixation treatment,

p_j = the fixed effect of the j^{th} percentage of added killed sperm,

$(tp)_{ij}$ = the fixed effect of the interaction of the i^{th} fixation treatment and the j^{th} percentage of added killed sperm,

$o_{k(ij)}$ = the random effect of the k^{th} observation nested within the ij^{th} fixation treatment-percentage added killed sperm subclass, and

e_{ijkl} = the error term.

Dependant variable % alive represented the average for 3 pairs of smears prepared from samples of each percent killed added-fixation treatment subclass where 200 sperm were randomly observed per smear. Data were transformed using the method $\sqrt{observations + .5}$. Method of transformation was selected based upon criteria proposed by Kirk (1982). Observation nested within percent by treatment was used to test treatment, percent killed sperm added and the interaction of treatment with percent. The error term tested observation nested within the treatment by percent subclass. Preplanned comparisons were performed between least squares means of fixation treatments within each percent killed added subclass (Appendix Table 1). Although data were transformed prior to analysis, least squares means of the untransformed data are reported but with significance based upon the results of analysis of the transformed data.

The second analysis of the Trial 2 data was preformed to determine if lines representing the means of percent killed added within each fixation treatment were the same (coincident). Multiple regression analysis using dummy variables (Kleinbaum and Kupper, 1978; Ott, 1984) was employed to simultaneously determine the slopes and intercepts of the lines representing the least squares means for fixed and unfixed samples using the model

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + e,$$

where

Y = the dependant variable % alive,

β_0 = the y-intercept for line representing unfixed sperm,

β_1 = the slope for line representing unfixed sperm,

x_1 = the percent killed sperm added,

β_2 = the difference between the y-intercepts of the lines representing unfixed sperm and fixed sperm,

x_2 = the dummy variable; equals 1 for fixed sperm, otherwise equals 0,

β_3 = the difference between the slopes of the lines representing fixed sperm and unfixed sperm, and

e = the error term.

Two GLM analyses of the Trial 2 data were performed using the SAS models $Y = x_1\ x_2\ x_1x_2$ (full model) and $Y = x_1$ (reduced model) whose members represent those effects previously described. The F value was determined using the formula

$$F = \frac{[SS_{(\text{Full model})} - SS_{(\text{Reduced model})}]\ /2}{ms_{\text{error(full model)}}}$$

and compared to the table F value having 2 numerator degrees of freedom and 80 error degrees of freedom (the error degrees of freedom of the full model). This F tested both differences between slopes and differences between intercepts of the lines representing fixed and unfixed sperm.

The data from Trial 3 was analyzed using the model

$$Y_{ijkl} = \mu + t_i + c_{j(i)} + s_k + ts_{(ik)} + e_{ijkl},$$

where

Y_{ijkl} = the individual observation for a given variable,

μ = the overall mean,

t_i = fixed effect of the i^{th} time post-insemination,

$c_{j(i)}$ = random effect of the j^{th} cow nested within the i^{th} time post-insemination,

s_k = the fixed effect of the k^{th} sperm sample,

$ts_{(ik)}$ = the fixed effect of the interaction of the i^{th} time post insemination and the k^{th} sperm sample, and

e_{ijkl} = the error term.

Dependant variable % live sperm represented the average for the pair of vital stained smears from each sample where 200 sperm were randomly counted per smear. Dependant variables representing the percentage of sperm in the morphological classifications (e.g. Normal, Abnormal, Primary, Secondary, Tertiary) were determined by randomly observing 200 sperm in wet mounts prepared from inseminate and uterine flush samples. The effect of time post-insemination was tested by cow nested within time post-insemination. The remaining effects were tested by the error term. Preplanned comparisons were performed between least squares means for samples within times post-insemination and across times post insemination for the viability and morphology variables (Appendix Table 1). Because too few sperm were recovered from two cows in each of the 4- and 8 hour post insemination sub-classes, their data was not included in the analyses.

Study 2

Two data sets were generated by this study which were analyzed using the model:

$$Y_{ijkl} = \mu + s_i + c_{j(i)} + l_k + sl_{(ik)} + e_{ijkl},$$

where

Y_{ijkl} = the individual observation for a given variable,

μ = the overall mean

s_i = the fixed effect of the i^{th} site of semen deposition

$c_{j(i)}$ = the random effect of the j^{th} cow nested within the i^{th} site of semen deposition,

l_k = the fixed effect of the k^{th} location of sample acquisition,

$sl_{(ik)}$ = the fixed effect of the interaction of the i^{th} site of semen deposition and the k^{th} location of sample acquisition, and

e_{ijkl} = the error term.

Dependant variables represented the percentage of 200 randomly observed sperm on a smear prepared from a sample obtained at each sample location in sperm trait classification (eg, live, normal, abnormal, live normal, live abnormal). The effect of semen deposition site was tested by cow nested within site. The remaining effects were tested by the error term.

In Experiment 1, uterine and inseminate data were analyzed using the data set comprised of inseminate, EYC incubation, uterine flush, retrograde mucus and vaginal mucus sperm data. Cervical data were analyzed as a data set separate from the above mentioned data. Because variances of cervical data were lower than those for data from the other locations, it was feared that inclusion of cervical data with the other data for analysis would have resulted in heterogeneity of variance. Data for retrograde and vaginal mucus were analyzed using the data set composed of inseminate, EYC incubation, retrograde mucus, vaginal mucus, and cervical data pooled across cervical quarters within the central and peripheral cervical regions.

In Experiment 2, uterine and inseminate data were analyzed using the data set comprised of inseminate, EYC incubation, uterine flush and retrograde mucus sperm data. Vaginal mucus data were not included in this data set because of the complete absence of data from one of the semen deposition sites, making least squares means and contrast sums of squares non-estimable by SAS. Cervical data were analyzed as in Experiment 1 for the same reasons as given for cervical data analysis in Experiment 1. Retrograde mucus data were analyzed using the same data set used for inseminate and uterine data. Vaginal mucus data was analyzed using the data set including all samples. However, vaginal insemination data was deleted from that data set for this analysis because non-estimable least-squares means and contrast sums of squares resulted with their inclusion. The vaginal mucus data were analyzed using the model:

$$Y_{ijk} = \mu + c_i + s_j + e_{ijk},$$

where

Y_{ijk} = the individual observation for a given variable,

μ = the overall mean,

c_i = the random effect of the ith cow,

s_j = the fixed effect of the jth sample location, and

e_{ijk} = the error term.

Dependant variables represented the percentage of 200 randomly observed sperm on a wet mount prepared from samples of the retrograde mucus in the sperm trait classifications (e.g. live, normal, live normal, live abnormal). Preplanned comparisons were performed among least squares means for location of sample recovery and between least squares means for site of semen deposition (Appendix Table 2). Chi-square analysis was performed on the results of the EYC-incubation in Study 1 and Study 2. Expected levels of live normal and live abnormal sperm were calculated using the observed values for each. The experimental Chi-square was then calculated and compared to the table chi-square having $\alpha = .05$ and 1 degree of freedom.

Results

Study 1 (Preliminary Studies)

The results of the validation of vital staining of fixed sperm (Trial 1 and Trial 2) are presented first, followed by the results of the preliminary sperm transport trial.

Trial 1

Analysis of variance showed that repetitions of the experiment in Trial 1 was not a significant source of variation for unstained sperm, but was significant for half-stained sperm and the combination of unstained with half stained. The time-fixation-kill treatment combination was a significant source of variation for unstained, half-stained and unstained plus half-stained sperm.

The results of Trial 1 are presented in Table 1 and Table 2. A comparison of the three replicates for fixed and unfixed semen prior to lethal treatment revealed significantly greater proportions of unstained sperm in fixed samples than in unfixed samples of semen (Table 1). Percentages of half-stained sperm, however, were significantly lower in fixed samples than in unfixed samples of semen and no significant differences existed among replicates for levels of unstained sperm or among levels of half-stained sperm in fixed samples of semen. Unfixed

Table 1. Least squares means ± standard errors for vital staining characteristics of unfixed and fixed bovine sperm prior to kill treatment. Trial 1.

Staining Characteristic	Replicate	Pre-Kill Treatment			
		Unfixed		Fixed	
% Unstained	1	63.2 ± 2.8		89.7 ± 3.3**	
	2	54.7 ± 2.8		87.9 ± 3.3**	
	3	39.5 ± 2.8[a]		85.3 ± 3.3**	
	mean		52.5 ± 1.6		87.6 ± 1.9
% Half Stained	1	20.6 ± 2.6[b]		4.1 ± 2.9**	
	2	27.4 ± 2.6[bc]		4.4 ± 2.9**	
	3	44.4 ± 2.6[c]		6.3 ± 2.9**	
	mean		30.8 ± 1.5		4.9 ± 1.8
% Unstained	1	83.7 ± 2.5		93.8 ± 2.9	
	2	82.1 ± 2.5		92.2 ± 2.9	
% Half Stained	3	83.9 ± 2.5		91.6 ± 2.9	
	mean		83.2 ± 1.4		92.5 ± 1.7

[a,b,c]Column means within staining characteristic having unlike superscripts differ ($p<.05$).
**means within lines differ ($p<.01$)
Note: contrasts defined in Appendix Table 1.

Table 2. Least squares means ± standard errors for vital staining characteristics of unfixed and fixed bovine sperm prior to kill treatment. Trial 1.

Staining Characteristic	Pre-kill Treatment		Kill Treatment	Post-kill Treatment	
	Unfixed	Fixed		Unfixed	Fixed
% Unstained	52.5 ± 1.6	87.6 ± 1.9	Heat	0.1 ± 2.8	0.1 ± 2.8
			Freeze	0.0 ± 2.8	0.0 ± 2.8
			Incubation	0.0 ± 2.8	0.1 ± 2.8
% Half Stained	30.8 ± 1.5	4.9 ± 1.8	Heat	1.9 ± 2.6	0.3 ± 2.6
			Freeze	0.0 ± 2.6	0.0 ± 2.6
			Incubation	1.2 ± 2.6	2.4 ± 2.6
% Unstained plus % Half Stained	83.2 ± 1.4	92.5 ± 1.7	Heat	2.0 ± 2.5	0.3 ± 2.5
			Freeze	0.0 ± 2.5	0.0 ± 2.5
			Incubation	1.2 ± 2.5	2.5 ± 2.5

Note: contrasts defined in Appendix Table 1

semen samples, on the other hand, showed significant differences among replicates for levels of unstained sperm and for levels of half-stained sperm. It is important to note that differences among vital staining results for unfixed and fixed sperm became nonsignificant when levels of unstained- and half-stained sperm were combined.

All kill treatments appeared equally effective and yielded very low levels of unstained (live) sperm (Table 2). In post-kill treatment samples, no significant differences in vital staining results were due to kill treatment or fixation method. This was true for unstained sperm, half-stained sperm and the combination of half-stained with unstained sperm.

These results indicate that the vital staining of unfixed and fixed samples was comparable regardless of the kill treatments. In fixed samples of semen before kill treatment, vital staining results in unstained and half-stained sperm appeared more uniform compared to those in the unfixed samples. Significant differences in vital staining between fixed and unfixed samples within kill treatment were eliminated when levels of unstained and half-stained sperm were combined. That result, coupled with significantly lower levels of half-stained sperm in fixed pre-kill treatment samples suggests that the half-stained sperm in the unfixed pre-kill treatment samples were the source of differences between vital staining results of unfixed vs fixed sperm. Thus, the results suggest that combination of half-stained sperm with unstained sperm yielded a truer picture of the level of live sperm in a population (fixed or unfixed) before, not as a result of, vital staining. The half-stained cells probably resulted from air-dry fixation which accompanies the vital staining process.

Trial 2

Analysis of variance showed that significant sources of variation for percentages of live sperm observed in Trial 2 were fixation treatment and percentage of killed sperm added. Both the interaction of fixation treatment with percentage of killed sperm added and observation nested within the interaction of fixation treatment with percentage of killed sperm added did not have significant effects on levels of live sperm observed.

The results of Trial 2 are summarized in Table 3. Based upon results of Trial 1, half-stained sperm were included with unstained sperm and counted as live sperm in this trial. Least squares means for percent live sperm in fixed semen samples were not significantly different from those in unfixed samples for semen treatments containing 0%, 10%, 20%, 40%, 60%, 80% or 100% added killed sperm. Multiple regression analysis using dummy variables (Kleinbaum, 1978; Ott, 1984) determined simultaneously that the slopes and intercepts of the lines representing least squares means for the fixed and unfixed semen samples in this Trial did not differ ($P>.10$). These coincident lines had a slope of $-.83 \pm .02$ and y-intercept of $89.01 \pm 1.25\%$ (figure 9).

The results of the two validation trials demonstrate that the results of eosin-aniline blue vital staining in unfixed bovine sperm are reproducible using the same semen preserved in a glutaraldehyde-paraformaldehyde fixative. This fixation method appeared to reduce the variation that occurred when vitally staining unfixed semen regardless of the method by which the cells were killed or the level of killed cells present, thus giving a more accurate picture of the sample's viability by apparently reducing some artifact introduced by the staining procedure. The artifact could have resulted from the variation in the length of time sperm were exposed to the vital stain, which is dependant upon thickness of the smear and the speed of air drying.

Trial 3

Two of the four cows in each of the 4- and 8 hour post-insemination groups were eliminated because too few sperm were recovered from their uteri for qualitative evaluation of that sperm population. Of the eight cows that remained in the Trial, 50% were known to have ovulated by the time of slaughter in the 4- and 12 hour post-insemination groups as evidenced by an ovulation site on an ovary. Neither of the cows in the 8 hour post-insemination group had ovulated. In all cows not showing evidence of ovulation, presence of at least one large follicle and absence of an active corpus luteum indicated they were in a periestrual condition. Standing estrus was observed in 3 of the 4 cows in the 12 hour post-insemination

Table 3. Least squares means ± standard errors for percent live sperm in fixed and unfixed semen samples to which graded levels of killed sperm were added. Study 1.

Fixation Treatment	Percent Killed Sperm Added						
	0%	10%	20%	40%	60%	80%	100%
Unfixed	84.4 ± 1.7	77.7 ± 1.7	70.8 ± 1.7	55.9 ± 1.7	41.8 ± 1.7	23.9 ± 1.7	0.0 ± 1.7
Fixed	86.8 ± 1.7	77.8 ± 1.7	74.5 ± 1.7	55.1 ± 1.7	47.5 ± 1.7	23.6 ± 1.7	0.0 ± 1.7

Note: contrasts defined in Appendix Table 1

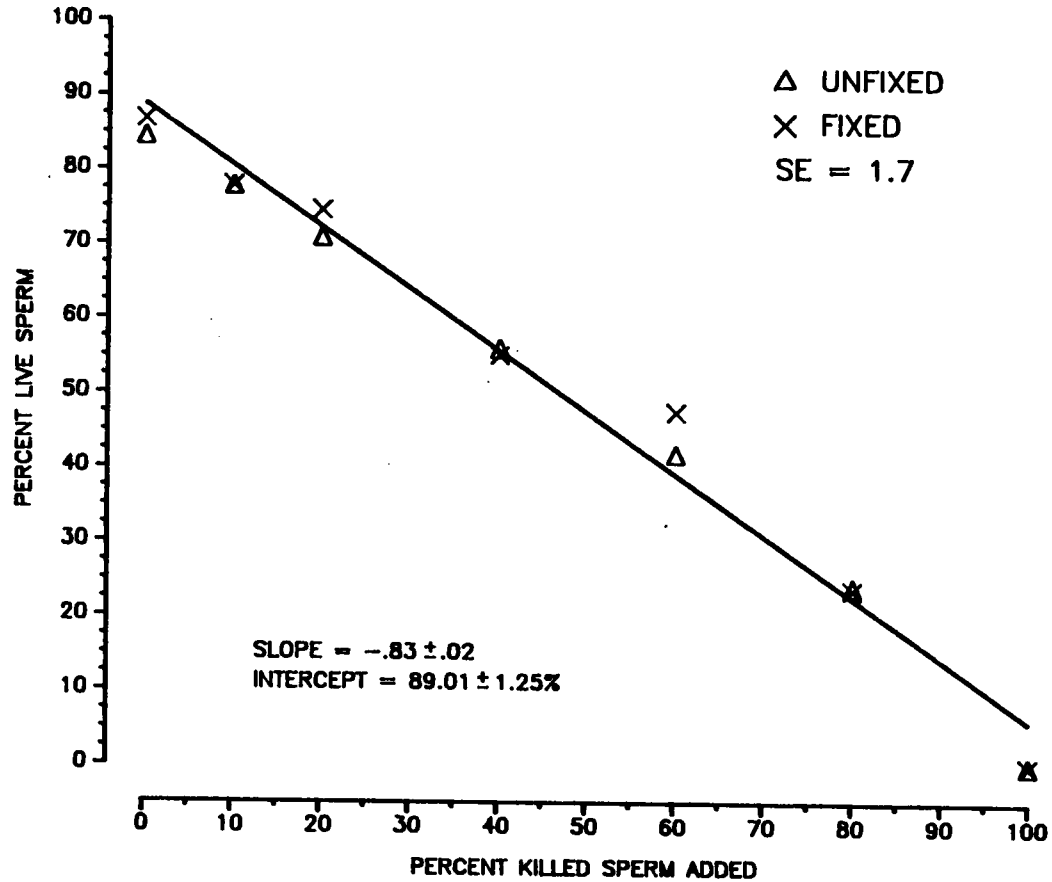

Figure 9. Vital staining results for unfixed and fixed sperm from Trial 2, after known proportions of killed sperm were added to unkilled sperm. Lines representing least squares means for unfixed and fixed sperm did not differ and are shown as coincident.

group and in each of the cows that remained in the 4- and 8 hour post-insemination groups. Mucus of normal estrus consistency and quantity was observed at breeding and in the vagina after tract excision in all cows except the one not showing standing estrus. The mean time ± standard deviation from breeding to slaughter was 4.25±0.0 hours, 8.13±.16 hours and 12.09±.14 hours for cows assigned to be sacrificed 4-, 8- and 12 hours post-insemination, respectively.

In Trial 3, analysis of variance showed that slaughter time post-insemination and cow nested within slaughter time post-insemination were not significant sources of variation for percentages of live sperm, normal sperm, or sperm having primary, secondary or tertiary abnormalities (Saacke and White, 1972). However, sample (inseminate or uterine flush) did have a significant effect on levels of live sperm, normal sperm and sperm having tertiary abnormalities, while percentages of sperm having primary or secondary abnormalities were not significantly affected by sample. The interaction of sample with time post-insemination had no significant effect on percentages of live sperm, normal sperm or sperm having primary, secondary or tertiary abnormalieis (Table 4). Thus, data were pooled across cows and times post-insemination (Table 5), resulting in significantly greater percentages of live sperm and morphologically normal sperm in the uterine flush population than in the inseminate. Proportions of sperm exhibiting tertiary (tail) abnormalities were significantly lower in the uterine flush than in the inseminate. Levels of sperm having primary (head) and secondary (droplet) abnormalities appeared to be lower (but not significantly) in the uterine flush as compared to the inseminate.

Table 6 summarizes percentages of the specific primary, secondary and tertiary sperm abnormalities found in the inseminate and uterine flush sperm populations pooled across cows and times post-insemination. Among the primary abnormalities, only the proportion of sperm having abnormal acrosomes was significantly lower in the uterine flush than in the inseminate. No significant differences existed between inseminate and uterine flush populations for levels of sperm having the remaining specific primary abnormalities. There was, however, a particular tendency toward reduced uterine levels of sperm having tapered heads

Table 4. Least squares means ± standard errors for percentages of sperm quality traits in the inseminates and uterine flushes 4-, 8-, and 12 h post-insemination (PI) pooled across cows. Study 1.

			Sperm Abnormalities		
Sample	% Live	% Normal	% Primary	% Secondary	% Tertiary
4 h PI					
Inseminate	69.0 ± 7.4	71.8 ± 5.5	13.3 ± 5.5	3.3 ± 4.9	11.0 ± 3.5
Uterine Flush	92.5 ± 7.4	88.8 ± 6.3	10.3 ± 5.5	1.0 ± 4.9	0.8 ± 3.5
8 h PI					
Inseminate	67.0 ± 7.4	58.5 ± 6.3	36.3 ± 5.5	3.0 ± 4.9	2.0 ± 3.6
Uterine Flush	91.5 ± 7.4	73.0 ± 6.3	23.3 ± 5.5	0.0 ± 4.9	0.8 ± 3.6
12 h PI					
Inseminate	87.9 ± 5.2	54.9 ± 4.4	21.7 ± 3.9	9.2 ± 3.5	12.2 ± 2.5
Uterine Flush	87.7 ± 5.2	83.8 ± 4.4	12.3 ± 3.9	0.2 ± 3.5	2.4 ± 2.5

Note: contrasts defined in Appendix Table 1

Table 5. Least squares means ± standard errors for percentages of sperm traits in the inseminate and uterine flush pooled across cows and times post-insemination. Study 1.

			Sperm Abnormalities		
Sample	% Live	% Normal	% Primary	% Secondary	% Tertiary
Inseminate	74.6 ± 3.9	61.7 ± 3.3	23.7 ± 2.9	5.1 ± 2.6	8.4 ± 1.9
Uterine Flush	90.5 ± 3.9*	81.1 ± 3.3**	15.4 ± 2.9	0.4 ± 2.6	1.3 ± 1.9*

*means within columns differ ($p<.05$)
**means within columns differ ($p<.01$)
Note: contrasts defined in Appendix Table 1.

and asymmetrically shaped heads. The effect of cow nested within time post-insemination was significant for tapered heads ($p = .01$) but not for asymmetric heads ($p = .62$). This significant effect on the former abnormality appeared to be due to one cow in the 8 hour post-insemination group (#1951). The inseminate and uterine flush from this cow contained 35.5% and 22.5% tapered-head sperm, respectively, while the levels of tapered-head sperm associated with the other experimental cows were much lower, ranging from $2.9 \pm 2.5\%$ to $9.0 \pm 2.5\%$.

Although percentages of sperm having secondary abnormalities were not significantly different between the inseminate and the uterine flush, uterine levels tended to be reduced compared to the already low levels in the inseminate (Table 6). A significant reduction in levels of sperm having coiled tails was observed in the uterine flush as compared to the inseminate, while levels of the other specific tertiary abnormalities appeared reduced, but not significantly so (Table 6).

The results of the preliminary trials showed that the uterine sperm population contained elevated levels of live sperm and normal sperm after vaginal semen deposition. These results suggested that live sperm and morphologically normal sperm either had a competitive advantage in reaching the uterus or were retained preferentially in the uterus. Uterine sperm numbers appeared to be adequate for qualitative definition of that population at 12 hours post-insemination. Thus, it was decided that subsequent studies would be conducted using 12 hours post insemination as the end point and using semen containing sperm having primarily abnormal head morphology and normal tail morphology. An additional reason for selecting 12 hours post-insemination over the other times, particularly 4 hours post-insemination, is the evidence in the literature that not until 8-10 hours post-insemination was there a build-up of sperm in the oviductal reservoir under sustained transport effects.

Table 6. Least squares means ± standard errors for percentages of specific sperm abnormalities in the inseminate and uterine flush pooled across cows and times post-insemination. Study 1.

	Primary (Head) Abnormalities				
Sample	% Tapered	% Asymmetric	% Cratered	% SFGS[a]	% Acrosome[b]
Inseminate	11.1 ± 1.3	10.8 ± 2.1	0.1 ± 0.1	1.0 ± 0.3	0.8 ± 0.1
Uterine Flush	7.6 ± 1.3	7.2 ± 2.1	0.2 ± 0.1	0.2 ± 0.3	0.1 ± 0.1*

	Secondary (Droplet) Abnormalities		
	% Proximal	% Translocating	% Distal
Inseminate	2.8 ± 2.0	2.1 ± 0.6	0.3 ± 0.1
Uterine Flush	0.2 ± 2.0	0.2 ± 0.6	0.0 ± 0.1

	Tertiary (Tail) Abnormalities		
	% Coiled	% Bent	% Abaxial
Inseminate	1.7 ± 0.4	0.2 ± 0.1	6.5 ± 1.8
Uterine Flush	0.1 ± 0.4*	0.0 ± 0.1	1.2 ± 1.8

[a]Percentage of sperm having short, flat, giant or slab-sided heads
[b]Percentage of sperm having ruffled, knobbed or incomplete acrosomes
*column means within abnormality differ ($p<.05$)
Note: contrasts defined in Appendix Table 1

Study 2

The results of this study are presented in two parts, with those of Experiment 1 followed by those of Experiment 2. Within each experiment, the results are reported in 6 parts: 1) general observations, 2) the inseminate and uterine sperm populations, 3) the cervical sperm population, 4) the retrograde mucus sperm population, 5) the vaginal mucus sperm population and 6) the EYC-incubated sperm.

Experiment 1.

To differentiate the effect of the cervix from that of the uterus on sperm quality in the uterus 12 hours post-insemination, high viability semen containing relatively high levels of primary abnormalities was used to inseminate 5 pairs of heifers. In one heifer of each pair, 5 ml semen was vaginally deposited and, as a control, .5 ml semen deposited in the uterus of the other heifer of each pair. Each pair of heifers manifested estrus behavior on the day of insemination as evidenced by triggered heat detectors and standing to be mounted by another heifer. All heifers showed low blood progesterone within 4 hours of insemination, as tested by an ELISA test for that hormone. At insemination, varying volumes of estrus mucus of normal appearance was expelled from the vulva of each heifer. The average time ± standard deviation (SD) from breeding to slaughter for vaginally inseminated heifers was 12.18±.45 hours and 12.18±.66 hours for intrauterine inseminated heifers. Mean time ± SD from slaughter until fixative was placed into the uterus was 22.0±11.5 minutes and 23.0±12.0 minutes for vaginally and intrauterine inseminated heifers, respectively. Semen was incubated in EYC for an average ± SD of 12.40±.23 hours prior to placing a sample in fixative. Inspection of the ovaries from each excised tract revealed that all heifers were in a periovulatory condition. Evidence of a recent ovulation was noted on the ovaries of 7 of the 10 heifers, while at least one large unruptured follicle and no evidence of an active corpus luteum

was observed on the ovaries from each of the remaining 3 heifers. Ovulation had occurred in at least one heifer of each pair.

One heifer was eliminated from the experiment due to an extreme uterine inflammatory response that was noted upon examination of the excised tract. The endometrium showed patchy areas of inflammation with small nodules of grey material protruding into the uterine lumen. Microscopic examination of the nodules showed them to be composed of neutrophils and monocytes. The tract was otherwise normal in appearance. This heifer had undergone intrauterine insemination and had ovulated. No such response was observed in the other heifer of that pair or in any other heifer, whether vaginally or intrauterine inseminated.

Histological survey of endometrial samples showed no sperm were found in any uterine gland profile in three of the vaginally inseminated heifers and two of the intrauterine inseminated heifers. Of the remaining heifers, the majority of uterine gland profiles contained no sperm. In those uterine gland profiles that contained sperm, a mean ± SD of 1.4 ± 0.9 sperm were observed per gland, which were usually found in the quarter of the gland closest to the uterine lumen. Based upon the histological survey, the uterine glands did not appear to harbor appreciable numbers of sperm after the uterus had been flushed, suggesting that failure to recover sperm from the uterine glands was not a significant source of error in this experiment.

The material recovered from the uteri of heifers in this experiment generally consisted of sperm, red blood cells, epithelial cells, leukocytes and an occasional piece of mucus. While leukocytes were observed in the uterine contents of each of the five intrauterine inseminated heifers, they were observed in the uteri from three of the five vaginally inseminated heifers.

Analysis of variance showed that site of semen deposition (vaginal, intrauterine) had no significant effect on percentages of any of the sperm traits in the uterus. Cow nested within site of semen deposition was a significant source of variation for percentages of all uterine sperm traits except for live sperm, decapitated sperm, live decapitated sperm and sperm having elongated heads. The effect of sample (inseminate, uterine flush) on percentages of

uterine sperm traits was significant only for live sperm, live normal sperm, abnormal sperm, live abnormal sperm, live primary abnormals and live tapered head sperm. The interaction of semen deposition site with sample was a significant source of variation only for percentages of live sperm and live sperm having tapered heads.

Percentages of sperm traits in the uterine sperm populations relative to the inseminates were not significantly different for both vaginal and intrauterine inseminated heifers (Table 7), neither were levels of sperm traits in the uterine populations different for vaginal vs intrauterine inseminated heifers. It is notable and surprising, however, that in most cases uterine semen deposition tended to result in a greater disparity between inseminate and uterine sperm quality than did vaginal insemination. There were no significant differences between inseminate and uterine levels of sperm with specific head abnormalities (Appendix Table 3) or of sperm both alive and having specific head abnormalities (Appendix Table 4) for either site of semen deposition although there appeared to be a tendancy toward reduced uterine levels of tapered-head after uterine deposition. Uterine levels of sperm having specific head abnormalities and of sperm both alive and having specific head abnormalities did not differ between sites of semen deposition.

The lack of difference between levels of uterine sperm population traits for vaginal vs intrauterine inseminated heifers, coupled with the lack of difference between inseminate and uterine levels of the sperm traits for the two sites of semen deposition, indicate that the cervix did not play a role in determining the quality of the uterine sperm population at 12 hours post-insemination. Alternatively, any effects the uterus may have had on the quality of the uterine sperm populatin could have been exerted by this time.

When data were pooled across sites of semen deposition, no significant differences existed between inseminate and uterine levels of live sperm (Table 8). It is important to point out that inseminate viability was high (i.e. 91% intact acrosomes) and uterine sperm viability was determined upon recovery 12 hours after insemination. There were, however, significantly greater proportions of normal sperm and significantly lower proportions of abnormal sperm and sperm having primary abnormalities in the uterus compared to the inseminate.

Table 7. Least squares means ± standard errors for percentages of sperm quality traits in the inseminate and uterine flush[a] sperm populations by semen deposition site.[b] Experiment 1.

Sperm Trait	Site	Inseminate	Uterine Flush
Live	v	91.9 ± 6.2	84.4 ± 6.2
	u	91.9 ± 6.2	85.9 ± 7.1
Normal	v	56.9 ± 3.0	61.5 ± 3.0
	u	56.9 ± 3.0	68.6 ± 3.5
Abnormal	v	44.2 ± 3.0	38.5 ± 3.0
	u	44.2 ± 3.0	31.8 ± 3.5
Primary[c]	v	43.0 ± 3.0	38.5 ± 3.0
	u	43.0 ± 3.0	31.4 ± 3.5
Live Normal	v	53.1 ± 4.8	52.8 ± 4.8
	u	53.1 ± 4.8	59.9 ± 5.6
Live Abnormal	v	39.0 ± 3.5	31.6 ± 3.5
	u	39.0 ± 3.5	26.4 ± 4.0
Live Primary[d]	v	38.1 ± 3.5	31.6 ± 3.5
	u	38.1 ± 3.5	26.0 ± 4.1

[a]Note: sperm quality 12 hours after insemination
[b]site: v = vaginal semen deposition
u = intrauterine semen deposition
[c]Percentage of sperm having head abnormalities
[d]Percentage of sperm both alive and having head abnormalities

Table 8. Least squares means ± standard errors for percentages of sperm quality traits in the inseminate and uterine flush[a] sperm populations pooled across semen deposition sites. Experiment 1.

Sperm Trait(%)	Inseminate	Uterine Flush
Live	91.1 ± 4.4	85.2 ± 4.7
Normal	56.9 ± 2.1	65.1 ± 2.3*
Abnormal	44.2 ± 2.1	35.2 ± 2.3*
Primary[b]	43.1 ± 2.1	34.9 ± 2.3*
Live Normal	53.1 ± 3.4	56.3 ± 3.7
Live Abnormal	39.0 ± 2.5	29.0 ± 2.7*
Live Primary[c]	38.1 ± 2.5	28.8 ± 2.7

[a]Note: sperm quality 12 hours after insemination
[b]Percentage of sperm having abnormal head morphology
[c]Percentage of sperm both alive and having abnormal head morphology
*means within lines differ ($p<.05$)

When the viability of the sperm in those morphological classifications was considered, differences in uterine and inseminate percentages of live normal sperm became nonsignificant while the significant differences remained between the inseminate and uterine sperm populations for percentages of live abnormal sperm. Differences between uterine and inseminate levels of sperm both alive and having primary abnormalities approached significance (contrast $F=6.30$ vs Bonferroni critical $F=6.50$) for reduced uterine levels. No significant differences existed between inseminate and uterine levels of sperm having specific head abnormalities or of sperm both alive and having specific head abnormalities (Appendix Table 5), however uterine levels tended to be reduced compared to the relatively low levels of specific abnormalities inseminated.

The viability of the semen used in this experiment was very high (e.g. 91% intact acrosomes) and was composed of high levels of viable normal and viable abnormal sperm. Of the abnormal sperm in this experiment, primary (head) abnormalities were almost exclusively evident, thus eliminating from consideration the effects that impaired motility associated with tail abnormalities are known to have on the transport of sperm in the female as a possible cause of reduced uterine levels of abnormal sperm. There was an insignificant decrease in sperm viability between the inseminate and the uterus (Table 7, Table 8) which was not unexpected in light of the 12 hours that had elapsed since insemination. Although sperm viability was probably necessary to cross the cervix to the uterus and to sustain sperm presence in the uterus, the viability of the uterine sperm populaiton was still quite high. Regardless, neither uterine sperm viability nor uterine sperm morphology (normal or abnormal) differed between the insemination sites (Table 7). When data were pooled across insemination sites, the uterine sperm population contained significantly greater proportions of normal sperm and significantly reduced levels of abnormal sperm and sperm having primary abnormalities, as compared to the inseminate (Table 8). Percentages of live normal sperm, however did not differ between the inseminate and the uterine population while live abnormals were significantly decreased and live primary abnormals approached significant reduction in the uterus. The insignificant (though apparent) decrease in levels of total live sperm in the uterus appears

to have negated the increased levels of normal uterine sperm, when their viability was considered, to yield a live normal uterine sperm population not different from the inseminate. The reduced levels of abnormal sperm in the uterus vs the inseminate (total abnormals and primary abnormals) did not appear affected by the change in total live sperm, resulting in the same relationship between inseminate and uterine sperm populations for levels of live abnormals and live primary abnormals as existed for the total abnormals and the primary abnormals. These results indicate that a subtle but real selection against morphologically abnormal sperm occurred between the time highly viable semen was inseminated and the recovery of sperm from the uterus. The lack of effect by site of semen deposition strongly indicates that selection was not due to a cervical effect alone.

Material recovered from the cervices generally consisted of mucus, epithelial cells, sperm and an occasional red blood cell. Not unexpectedly, epithelial cells were much more prevalent in vacuum samples than in curettage samples.

Analysis of variance showed that the site of semen deposition was a significant source of variation only for percentages of decapitated sperm and live decapitated sperm in the cervix. The effect of cow nested within site of semen deposition was significant only for percentages of live sperm, normal sperm, abnormal sperm, primary abnormals, live normal sperm and flat-head sperm. Sample location did have a significant effect on cervical levels of live sperm, normal sperm, tapered head sperm and live short-headed sperm. The interaction of insemination site with sample was shown by analysis of variance to be a non-significant source of variation for percentages of all sperm traits in the cervix. For both vaginal and intrauterine inseminated heifers, no significant differences existed in percentages of any sperm trait from one cervical quarter to the next within cervical region (central or peripheral) or between regions (central vs peripheral) within quarters of the cervix for vaginal or intrauterine inseminated heifers (Appendix Table 6, 7, 8). Neither were there significant differences between vaginal and intrauterine inseminated heifers for percentages of any sperm trait among cervical quarters within region or between regions within cervical quarters.

There was also no effect of cervical quarter on levels of any sperm trait when data were pooled across semen deposition sites. No significant differences existed among cervical quarters or between regions of the cervix (central vs peripheral) within cervical quarters for levels of any sperm trait (Appendix Table 9, 10).

When data were pooled across cervical quarters and insemination sites, significantly greater proportions of live sperm and live normal sperm were found in the peripheral vs the central cervix (Table 9). Although proportions of normal sperm, live abnormal sperm and sperm both alive and having primary abnormalities (live primary abnormals) tended to be elevated in peripheral vs central cervical regions, the differences were not significant. For sperm having specific head abnormalities, only significantly lower levels of sperm with tapered heads and sperm both alive and having short heads were found in the peripheral vs central cervical regions (Appendix Table 11).

These results suggest the existence of a gradient of viable sperm within the cervix. A sperm population of higher viability existed deeper within the folds of the cervical mucosa than at the luminal aspect of those folds and was composed of a greater proportion live normal sperm than the central cervical population. This implies preferential habitation of the region between the cervical folds by live sperm and live normal sperm.

Analysis of variance showed that site of semen deposition had no significant effect on percentage of any sperm trait in the vaginal mucus, with the exception of live decapitated sperm. Cow nested within site of semen deposition was a significant source of variation for percentages of all vaginal mucus sperm traits except decapitated sperm, live decapitated sperm and live elongated head sperm. The effect of sample was significant only on proportions of live sperm, normal sperm, live normal sperm, primary abnormals, live primary abnormals, total abnormals, total live abnormals, pyriform-head sperm, live tapered head sperm, elongated head sperm, live elongated head sperm and live cratered head sperm. The interaction of semen deposition site with sample location was a significant source of variation only for percentages of live sperm and live tapered head sperm in the vaginal mucus.

Table 9. Least squares means ± standard errors for percentages of sperm quality traits in the cervical sperm population by cervical region[a] and pooled across cervical quarters and semen deposition. Experiment 1.

Sperm Trait	Cervical Region	
	Central	Peripheral
Live	65.2 ± 2.7	77.4 ± 2.2*
Normal	60.4 ± 1.5	63.5 ± 1.2
Abnormal	39.7 ± 1.5	36.5 ± 1.2
Primary[b]	39.6 ± 1.5	36.5 ± 1.2
Live Normal	40.4 ± 1.4	50.3 ± 1.1**
Live Abnormal	24.9 ± 2.1	27.2 ± 1.7
Live Primary[c]	24.8 ± 2.1	27.2 ± 1.7

[a]Cervical region: Central: obtained by curettage of cervical mucosa proximal to cervical canal
Peripheral: obtained by vacuum deeper between 1° and 2° folds of cervical mucosa

[b]Percentage of sperm having abnormal head morphology

[c]Percentage of sperm both alive and having abnormal head morphology

*Means within lines differ ($p<.05$)

**Denotes means within lines differ ($p<.01$)

No significant differences existed between vaginal and intrauterine-inseminated heifers for percentages of any sperm trait in the vaginal mucus (Table 10, Appendix Tables 12, 13). There were, however, significantly lower percentages of live sperm in the vaginal mucus than in the inseminate for both insemination sites (Table 10). Proportions of live sperm in the vaginal mucus were significantly lower than in the uterine population for intrauterine-inseminated heifers and tended to be lower in the vaginal mucus for vaginally-inseminated heifers. This suggests that retrograde removal of nonviable sperm from the uterus after intrauterine insemination was of greater magnitude than it was after vaginal insemination at the time of sampling. This could have occurred because many nonviable sperm may have been removed prior to their arrival in the uterus after vaginal insemination.

When data were pooled across sites of semen deposition, levels of live sperm and live normal sperm were greater in the inseminate, peripheral cervix and uterine sperm populations than in the vaginal mucus (Table 11). Proportions of normal sperm, abnormal sperm, and sperm with primary abnormalities in the inseminate, cervix and uterus did not differ from those in the vaginal mucus. Although proportions of live abnormal sperm and live primary abnormals were significantly reduced in the vaginal mucus vs the inseminate, no significant differences existed with other sample locations.

For the most part, percentages of sperm having specific head abnormalities and percentages of sperm both alive and having specific head abnormalities in the vaginal mucus were not significantly different from those in the other sample locations (Table 12). Levels of live sperm with tapered heads, however, were significantly lower in the vaginal mucus than in the inseminate but did not differ from the other tract locations.

These data show that the sperm population in the vaginal mucus closely resembled that of the rest of the tract and the inseminate when sperm morphology alone was considered (Table 11, Table 12). On the other hand, when sperm viability alone was considered (i.e. total live sperm, Table 11) the vaginal mucus sperm population resembled only the population in the central cervical region. This was also true for live normal sperm in the vaginal mucus. When the viability of the abnormal sperm was considered (i.e. live abnormals and live primary

Table 10. Least squares means ± standard errors for percentages of sperm quality traits in the vaginal mucus sperm population compared to those in other sample locations by insemination site.[a] Experiment 1.

Sperm Trait	Site	Vaginal Mucus	Inseminate	Cervix		Uterus
				Central	Peripheral	
Live	v	54.2 ± 7.8	91.1 ± 5.8*	66.6 ± 6.7	77.4 ± 6.7	84.4 ± 5.8
	u	42.0 ± 7.9	91.1 ± 5.8**	53.0 ± 9.8	71.4 ± 7.9	85.9 ± 6.8**
Normal	v	56.0 ± 3.6	56.9 ± 2.7	60.8 ± 3.1	64.2 ± 3.1	61.5 ± 2.8
	u	59.9 ± 3.7	56.9 ± 2.7	61.6 ± 4.6	65.5 ± 3.7	68.6 ± 3.2
Abnormal	v	43.7 ± 3.7	44.2 ± 2.8	39.6 ± 3.1	36.1 ± 3.1	38.5 ± 2.8
	u	40.9 ± 3.7	44.2 ± 2.8	39.3 ± 4.6	35.5 ± 3.7	31.8 ± 3.2
Primary	v	44.0 ± 3.7	43.1 ± 2.8	39.2 ± 3.1	35.8 ± 3.1	38.5 ± 2.8
	u	40.1 ± 3.7	43.1 ± 2.8	38.4 ± 4.6	34.5 ± 3.7	31.4 ± 3.2
Live Normal	v	30.8 ± 6.1	53.1 ± 4.6	41.3 ± 5.2	50.6 ± 5.2	52.8 ± 4.6
	u	21.8 ± 6.2	53.1 ± 4.6	33.3 ± 7.7	47.3 ± 6.2	59.9 ± 5.3
Live Abnormal	v	23.2 ± 4.3	39.0 ± 3.2	25.7 ± 3.6	27.1 ± 3.6	31.6 ± 3.2
	u	20.3 ± 4.3	39.0 ± 3.2	20.4 ± 5.3	24.8 ± 4.3	26.4 ± 3.7
Live Primary	v	23.4 ± 4.3	38.1 ± 3.2	25.3 ± 3.7	26.8 ± 3.7	31.6 ± 3.2
	u	20.3 ± 4.4	38.1 ± 3.2	19.7 ± 5.4	24.0 ± 4.4	26.0 ± 3.7

[a]Insemination site v = vaginal semen deposition
u = intrauterine semen deposition

*Differs from vaginal mucus mean ($p<.05$)

**Differs from vaginal mucus mean ($p<.01$)

Table 11. Least squares means ± standard errors for percentages of sperm traits in the vaginal mucus sperm population compared to those in other sample locations pooled across insemination site. Experiment 1.

Sperm Trait	Vaginal Mucus	Inseminate	Cervix[a]		Uterus
			Central	Peripheral	
Live	48.1 ± 5.6	91.1 ± 4.1**	59.8 ± 5.9	74.4 ± 5.2*	85.2 ± 4.5**
Normal	58.0 ± 2.6	56.9 ± 1.9	61.2 ± 2.8	64.8 ± 2.4	65.0 ± 2.1
Abnormal	42.3 ± 2.6	44.2 ± 1.9	39.4 ± 2.8	35.8 ± 2.4	35.2 ± 2.1
Primary[b]	42.0 ± 2.6	43.1 ± 1.9	38.8 ± 2.8	35.2 ± 2.4	34.9 ± 2.1
Live Normal	26.3 ± 4.4	53.1 ± 3.2**	37.3 ± 4.6	48.9 ± 4.0**	56.3 ± 3.5**
Live Abnormal	21.8 ± 3.0	39.0 ± 2.2**	23.0 ± 3.2	26.0 ± 2.8	29.0 ± 2.4
Live Primary[c]	21.8 ± 3.1	38.1 ± 2.3**	22.5 ± 3.3	25.4 ± 2.9	28.8 ± 2.5

[a]Central cervix = obtained by curettage of cervical mucosa proximal to cervical canal
Peripheral cervix = obtained by vacuum deeper between 1° and 2° folds of cervical mucosa
[b]Percentage of sperm having abnormal head morphology
[c]Percentage of sperm both alive and having abnormal head morphology
*Differs from vaginal mucus mean ($p<.05$)
**Differs from vaginal mucus mean ($p<.01$)

Table 12. Least squares means ± standard errors for percentages of sperm head abnormalities in the vaginal mucus sperm population compared to those in other sample locations pooled across insemination site. Experiment 1.

Sperm Trait	Vaginal Mucus	Inseminate	Cervix[a]		Uterus
			Central	Peripheral	
Tapered	23.4 ± 1.9	23.4 ± 1.4	23.4 ± 2.1	20.5 ± 1.8	19.7 ± 1.5
Cratered	7.7 ± 0.8	9.2 ± 0.6	7.2 ± 0.9	6.8 ± 0.7	7.8 ± 0.6
Asymmetric	6.1 ± 1.0	6.0 ± 0.7	5.0 ± 1.1	5.0 ± 0.9	4.9 ± 0.8
Decapitated	3.4 ± 0.9	1.6 ± 0.6	2.3 ± 0.9	2.0 ± 0.8	1.4 ± 0.7
Pyriform	0.3 ± 0.2	0.7 ± 0.2	0.1 ± 0.2	0.0 ± 0.2	0.2 ± 0.2
Short	0.4 ± 0.2	0.5 ± 0.1	0.5 ± 0.2	0.1 ± 0.2	0.2 ± 0.1
Flat	0.1 ± 0.2	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.6 ± 0.1
Elongated	0.0 ± 0.1	0.0 ± 0.04	0.1 ± 0.1	0.2 ± 0.1*	0.0 ± 0.04
Live Tapered	11.5 ± 1.9	21.3 ± 1.5**	14.3 ± 2.1	15.0 ± 1.8	16.4 ± 1.6
Live Cratered	5.7 ± 1.2	8.6 ± 0.9	3.3 ± 1.3	4.8 ± 1.1	6.6 ± 1.0
Live Asymmetric	2.9 ± 0.9	5.4 ± 0.7	3.2 ± 0.9	4.1 ± 0.8	4.0 ± 0.7
Live Decapitated	0.5 ± 0.3	0.4 ± 0.3	1.0 ± 0.4	1.1 ± 0.3	0.9 ± 0.3
Live Pyriform	0.3 ± 0.2	0.5 ± 0.2	0.1 ± 0.2	0.0 ± 0.2	0.1 ± 0.2
Live Short	0.2 ± 0.2	0.5 ± 0.1	0.5 ± 0.2	0.0 ± 0.1	0.1 ± 0.1
Live Flat	0.0 ± 0.2	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.2	0.6 ± 0.1
Live Elongated	0.0 ± 0.1	0.0 ± 0.04	0.1 ± 0.1	0.2 ± 0.1*	0.0 ± 0.04

[a]Central cervix = obtained by curettage of cervical mucosa proximal to cervical canal
Peripheral cervix = obtained by vacuum deeper between 1° and 2° folds of cervical mucosa
*Differs from vaginal mucus mean ($p<.05$)
**Differs from vaginal mucus mean ($p<.01$)

abnormals) the vaginal mucus reflected levels in the other tract locations but was reduced compared to the inseminate. These results suggest that the presence of sperm in the vaginal mucus was related primarily to their viability, presumably originating from the central cervix, and probably a result of retrograde removal.

The site of semen deposition was shown by analysis of variance to be a significant source of variation only for percentages of live decapitated sperm in the retrograde mucus. The effect of cow nested within site of semen deposition was significant for percentages of all sperm traits in the retrograde mucus, with the exception of decapitated sperm, live decapitated sperm and live elongated head sperm. Sample location was shown to have a significant effect on percentages of live sperm, normal sperm, live normal sperm, primary abnormals, live primary abnormals, total abnormals, total live abnormals, pyriform head sperm, live tapered head sperm, elongated head sperm, live elongated head sperm and live cratered head sperm in the retrograde mucus. The interaction of semen deposition site with sample location only had a significant effect on vaginal mucus percentages of live sperm and live tapered head sperm.

The only significant differences that existed between vaginal and intrauterine inseminated heifers for percentages of the sperm traits in the retrograde mucus were for levels of live sperm (Table 13) and live sperm having tapered heads (Appendix Table 14), both of which were reduced in the retrograde mucus of intrauterine-inseminated heifers. Levels of those two sperm traits in the retrograde mucus of vaginally inseminated heifers were not significantly different from those in the other samples. For intrauterine inseminated heifers, proportions of live sperm in the retrograde mucus reflected only the cervical sperm population (Table 13) while levels of live sperm with tapered heads differed only from the inseminate population (Appendix Table 14). Percentages of all other sperm traits in the retrograde mucus did not differ from those in other sample locations (Table 12, Appendix Table 14, 15). These retrograde mucus results verify the wastage of viable sperm that occurs following vaginal as opposed to uterine semen deposition.

Table 13. Least squares means ± standard errors for percentages of sperm quality traits in the retrograde mucus sperm population compared to those in other sample locations, by insemination site.[a] Experiment 1.

Sperm Trait	Site	Retrograde Mucus	Inseminate	Cervix		Uterus
				Central	Peripheral	
Live	v	83.7 ± 5.8	91.1 ± 5.8	66.6 ± 6.7	77.4 ± 6.7	84.4 ± 5.8
	u	43.7 ± 6.7^{++}	91.1 ± 5.8**	53.0 ± 9.8	71.4 ± 7.9	85.9 ± 6.8**
Normal	v	58.6 ± 2.8	56.9 ± 2.7	60.8 ± 3.1	64.2 ± 3.1	61.5 ± 2.8
	u	63.2 ± 3.2	56.9 ± 2.7	61.6 ± 4.6	65.5 ± 3.7	68.6 ± 3.2
Abnormal	v	41.8 ± 2.8	44.2 ± 2.8	39.6 ± 3.1	36.1 ± 3.1	38.5 ± 2.8
	u	37.6 ± 3.2	44.2 ± 2.8	39.3 ± 4.6	35.5 ± 3.7	31.8 ± 3.2
Primary[b]	v	41.4 ± 2.8	43.1 ± 2.8	39.2 ± 3.1	35.8 ± 3.1	38.5 ± 2.8
	u	36.8 ± 3.2	43.1 ± 2.8	38.4 ± 4.6	34.5 ± 3.7	31.4 ± 3.2
Live Normal	v	49.6 ± 4.6	53.1 ± 4.6	41.3 ± 5.2	50.6 ± 5.2	52.8 ± 4.6
	u	27.6 ± 5.2	53.1 ± 4.6	33.3 ± 7.7	47.3 ± 6.2	59.9 ± 5.3
Live Abnormal	v	34.3 ± 3.2	39.0 ± 3.2	25.7 ± 3.6	27.1 ± 3.6	31.6 ± 3.2
	u	16.8 ± 3.6	39.0 ± 3.2	20.4 ± 5.3	24.8 ± 4.3	26.4 ± 3.7
Live Primary[c]	v	34.1 ± 3.2	38.1 ± 3.2	25.3 ± 3.7	26.8 ± 3.7	31.6 ± 3.2
	u	16.1 ± 3.7	38.1 ± 3.2	19.7 ± 5.4	24.0 ± 4.4	26.0 ± 3.7

[a]Insemination site v = vaginal semen deposition
u = intrauterine semen deposition
[b]Percentage of sperm having head abnormalities
[c]Percentage of sperm both alive and having head abnormalities
[**]Differs from retrograde mucus mean ($p<.01$)
[++]Column means within sperm trait differ ($p<.01$)

When results were pooled across insemination sites, levels of normal sperm, abnormal sperm and sperm with primary abnormalities in the retrograde mucus were not significantly different from those in the other sample locations (Table 14). Levels of live abnormal sperm and levels of live sperm with primary abnormalities in the retrograde mucus were significantly different from only those levels in the inseminate.

In general, retrograde mucus levels of sperm having specific head abnormalities and of live sperm having specific head abnormalities did not differ significantly from those in other sample locations (Appendix Table 16). Cratered sperm and live cratered sperm exhibited significantly reduced levels in the retrograde mucus as compared to the inseminate but did not differ from levels in the other sample locations. Levels of sperm having pyriform heads were significantly lower in both cervical locations than in the retrograde mucus. Live sperm with pyriform heads were significantly reduced in the uterine sperm population as compared to the retrograde mucus.

These results indicate that the sperm population in the retrograde mucus, as with the vaginal mucus, closely resembled those in other tract locations and the inseminate in terms of morphology. Further, these data show that the presence of sperm in the retrograde mucus of intrauterine inseminated heifers was based upon the viability of those sperm, while for vaginally inseminated heifers, sperm presence in the retrograde mucus appeared related to neither sperm morphology nor sperm viability. These results indicate that the retrograde mucus acted as a vehicle for the removal of nonviable sperm, regardless of their morphology, from the reproductive tracts of these heifers.

Table 15 shows the results of *in vitro* incubation of inseminate samples. Chi-square analysis showed that the changes in level of live normal sperm did not significantly differ from that for live abnormal sperm. This result indicates that differential death of the normal vs abnormal sperm in the semen used in this experiment neither occurred *in vitro* nor, presumably, *in vivo*.

Table 14. Least squares means ± standard errors for percentages of sperm quality traits in the retrograde mucus sperm population compared to those in other sample locations pooled across insemination site. Experiment 1.

Sperm Trait	Retrograde Mucus	Inseminate	Cervix[a]		Uterus
			Central	Peripheral	
Live	63.7 ± 4.4	91.1 ± 4.1**	59.8 ± 5.9	74.4 ± 5.2	85.2 ± 4.5**
Normal	60.9 ± 2.1	56.9 ± 1.9	61.2 ± 2.8	64.8 ± 2.4	65.0 ± 2.1
Abnormal	39.7 ± 2.1	44.2 ± 1.9	39.4 ± 2.8	35.8 ± 2.4	35.2 ± 2.1
Primary[b]	39.1 ± 2.1	43.1 ± 1.9	38.8 ± 2.8	35.2 ± 2.4	34.9 ± 2.1
Live Normal	38.6 ± 3.5	53.1 ± 3.2*	37.3 ± 4.6	48.9 ± 4.0	56.3 ± 3.5**
Live Abnormal	25.5 ± 2.4	39.0 ± 2.2**	23.0 ± 3.2	26.0 ± 2.8	29.0 ± 2.4
Live Primary[c]	25.1 ± 2.5	38.1 ± 2.3**	22.5 ± 3.3	25.4 ± 2.9	28.8 ± 2.5

[a]Central cervix = obtained by curettage of cervical mucosa proximal to cervical canal
Peripheral Cervix = obtained by vacuum deeper between 1^{o} and 2^{o} folds of cervical mucosa
[b]Percentage of sperm having abnormal head morphology
[c]Percentage of sperm both alive and having abnormal head morphology
*Differs from retrograde mucus mean ($p<.05$)
**Differs from retrograde mucus mean ($p<.01$)

Table 15. Least squares means ± standard errors for percentages of live normal sperm[a] and live abnormal sperm[b] in samples before and after *in vitro* incubation.[c] Experiment 1.

	Incubation[c]		
Sperm Trait	Before	After	Change
Live Normal[a]	53.1 ± 3.4	39.9 ± 3.4	-13.2
Live Abnormal[b]	39.0 ± 2.5	32.6 ± 2.5	-6.4

[a]Percentage of sperm both alive and having normal morphology
[b]Percentage of sperm both alive and having abnormal morphology
[c]Semen incubated 12.4 ± .23 hours to coincide with time between deposition and recovery of sperm in the tract

Experiment 2.

This experiment utilized 3 pairs of heifers, the members of each being vaginally or intrauterine inseminated with low viability semen (i.e. 32% intact acrosomes) as a contrast for the high viability semen used in Experiment 1. On the day of insemination, heifers in each pair showed estrus behavior as evidenced by triggered heat detectors and willingness to stand to be mounted. The ELISA test for blood progesterone, which was employed within 4 hours prior to insemination yielded low levels for that hormone in all heifers. Estrus mucus of normal appearance and consistency was expelled upon insemination of 2 of the 3 pairs of heifers. No mucus was expelled upon insemination from either heifer in one pair. The average time ± SD from insemination to slaughter for vaginally inseminated heifers was 11.53±.29 hours and 11.67±.14 hours for intrauterine inseminated heifers. Mean time ± SD from slaughter until fixative was placed in the uterus was 23.3±5.8 minutes and 18.3±.29 minutes for vaginally and intrauterine inseminated heifers, respectively. Semen was incubated in EYC for an average ± SD of 11.72±.13 hours prior to placing a sample in fixative. Inspection of the ovaries of the excised tracts revealed that all heifers were in a peri-ovulatory state. Evidence of a recent ovulation was noted on an ovary from each heifer in two of the pairs. Neither heifer in the remaining pair had ovulated; however, at least one large follicle and no functional corpora lutea were noted in either case.

Histological sections prepared from endometrium after uteri were flushed with fixative rarely exhibited uterine glands containing sperm and then only an average ± SD of 1.6±1.1 sperm were found per gland. These findings verify that sperm recovery from the uterus was quite complete and did not include any apparent select population of retained sperm.

Analysis of variance showed that semen deposition site was not a significant source of variation for percentages of any sperm trait in the uterus. For percentages of total live abnormals and live primary abnormals, however, semen deposition site approached significance ($p=.06$ in each case). Cow nested within site of semen deposition had a significant effect only on proportions of live sperm, pyriform head sperm, live pyriform head sperm, tapered head sperm, decapitated sperm, flat head sperm and live flat head sperm in the uterus.

The effect of sample (inseminate, uterus) was significant on percentages of live sperm, live normal sperm, total live abnormal sperm, live primary abnormals, live tapered head sperm and flat head sperm in the uterus. The interaction of semen deposition site with sample was a significant source of variation for uterine proportions of live sperm, live normal sperm, total live abnormal sperm, live primary abnormals and pryiform head sperm.

No significant difference existed between the insemination sites for uterine percentages of live sperm, normal sperm, or total abnormal sperm (Table 16). Similarly, uterine levels for live normal sperm were not significantly different between the two insemination sites. Significantly greater percentages of live abnormals were found in the uterine population of vaginally vs intrauterine inseminated heifers. Although most uterine proportions of sperm having specific head abnormalities did not differ significantly between the two insemination sites, a notable trend towards increased uterine proportions of sperm having tapered heads and significantly greater uterine proportions of sperm both alive and having tapered heads was apparent in vaginally inseminated heifers (Appendix Table 17 and Appendix Table 18, respectively).

In this experiment, the uterine sperm population differed from the inseminate population primarily in terms of sperm viability. Significantly greater proportions of live sperm and live normal sperm were found in the uterine vs inseminate populations for both vaginally and intrauterine-inseminated heifers (Table 16). While proportions of live abnormals in the uterine population of vaginally inseminated heifers was greater than that in the inseminate, no differences existed between the two populations for those traits in intrauterine inseminated heifers. No significant differences existed between the inseminate and uterine sperm populations for percentages of sperm having specific head abnormalities (Table 17) or for levels of sperm both alive and having specific head abnormalities (Appendix Table 17). Of the sperm having specific head abnormalities, there was a distinct tendancy for the relatively high inseminate levels of decapitated sperm (12.3%) to be reduced in the uterine population (1.0%-4.3%) regardless of insemination site (Table 17). This result is explained by the fact that such sperm have no flagellar ability and thus may be readily excluded from the tract. In

Table 16. Least squares means ± standard errors for percentages of sperm quality traits in the inseminate and uterine flush[a] sperm populations by semen deposition site.[b] Experiment 2.

Sperm Trait	Site	Inseminate	Uterine Flush
Live	v	32.1 ± 4.9**	83.5 ± 4.9
	u	32.1 ± 4.9**	71.4 ± 4.9
Normal	v	71.2 ± 7.0	67.0 ± 7.0
	u	71.2 ± 7.0	76.2 ± 7.0
Abnormal	v	28.8 ± 7.0	33.0 ± 7.0
	u	28.8 ± 7.0	23.8 ± 7.0
Live Normal	v	23.3 ± 4.0**	56.1 ± 4.0
	u	23.3 ± 4.0**	56.9 ± 4.0
Live Abnormal	v	8.8 ± 2.9**	27.5 ± 2.9++
	u	8.8 ± 2.9	14.5 ± 2.9

[a]Note: sperm quality 12 hours after insemination
[b]Insemination site v = vaginal semen deposition
u = intrauterine semen deposition
**means within rows differ (p<.01)
++column means within sperm trait differ (p<.01)

Table 17. Least squares means ± standard errors for percentages of sperm head abnormalities in the inseminate and uterine flush sperm populations by semen deposition site.[a] Experiment 2.

Sperm Trait	Site	Inseminate	Uterine Flush
Tapered	v	7.2 ± 2.1	16.9 ± 2.1
	u	7.2 ± 2.1	9.2 ± 2.1
Decapitated	v	12.3 ± 5.7	1.0 ± 5.7
	u	12.3 ± 5.7	4.3 ± 5.7
Flat	v	4.0 ± 1.0	3.3 ± 1.0
	u	4.0 ± 1.0	3.3 ± 1.0
Elongated	v	3.5 ± 1.3	4.2 ± 1.3
	u	3.5 ± 1.3	3.3 ± 1.3
Cratered	v	3.3 ± 0.7	2.8 ± 0.7
	u	3.3 ± 0.7	3.5 ± 0.7
Pyriform	v	1.3 ± 0.2	1.0 ± 0.2
	u	1.3 ± 0.2	0.7 ± 0.2
Asymmetric	v	0.5 ± 0.9	2.8 ± 0.9
	u	0.5 ± 0.9	0.2 ± 0.9
Short	v	0.5 ± 0.3	0.2 ± 0.3
	u	0.5 ± 0.3	0.0 ± 0.3

[a]site v = vaginal semen deposition
u = intrauterine semen deposition

addition, nearly all decapitated sperm were dead (Appendix Table 17), showing very low, but comparable levels between the inseminate and uterine populations. This appears to have played a role in the significantly greater proportions of uterine vs inseminate live abnormals after vaginal deposition and the apparent (but nonsignificant) increase after uterine deposition (Table 16). Uterine levels of sperm having tapered heads and of sperm both alive and having tapered heads were notably, but not significantly, elevated in vaginally inseminated heifers (Table 17 and Appendix Table 17, respectively).

The overall similarity in percentages of sperm traits between the uterine populations for the two insemination sites indicates that the cervix had little qualitative effect on the uterine sperm population. The enrichment in the viability of the uterine sperm population compared to that of the inseminate, coupled with the striking similarity between the sperm morphological profiles of inseminate vs uterine populations point to a shift based upon sperm viability (or motile ability, as illustrated by decapitated sperm), not morphology *per se*.

When data were pooled across sites of semen deposition, the trend toward enriched viability of the uterine sperm population was maintained. A significantly greater proportion of live, live normal and live abnormal sperm existed in the uterine vs inseminate populations (Table 18). Percentages of normal sperm and abnormal sperm were not significantly different between the inseminate and uterine populations. The proportions of sperm having specific head abnormalities did not differ significantly between the inseminate and uterine populations, an effect that was also generally true for sperm both alive and having specific head abnormalities (Appendix Table 18), with the exception of tapered head sperm. For this abnormality, significantly elevated uterine percentages of live tapered head sperm and an apparent, though nonsignificant, increase in uterine levels of sperm having tapered heads (total tapers) than in the inseminate.

As in Experiment 1, these results indicate that a qualitative selection of sperm occurred between the time of semen deposition and the time of sperm recovery from the uterus. The lack of difference due to site of semen deposition (Table 17) suggests that the cervix played an insignificant role in determining the quality of the uterine sperm population at the

Table 18. Least squares means ± standard errors for percentages of sperm quality traits in the inseminate and uterine flush[a] sperm populations pooled across semen deposition sites. Experiment 2.

Sperm Trait	Inseminate	Uterine Flush
Live	32.1 ± 3.5	77.5 ± 3.5**
Normal	71.2 ± 5.0	71.6 ± 5.0
Abnormal	28.8 ± 5.0	28.4 ± 5.0
Live Normal	23.3 ± 2.8	56.5 ± 2.8**
Live Abnormal	8.8 ± 2.0	21.0 ± 2.0**

[a]Note: sperm quality 12 hours after insemination
**means within lines differ ($p<.01$).

time of sampling. Differences in the proportions of viable sperm between the inseminate and uterine populations (Table 16) and the lack of differences in proportions of sperm morphology traits between the two samples (Table 17) indicate that sperm selection was based upon the viability and not the morphology of the gametes. These indications are reinforced by the enriched uterine levels of live abnormal sperm in vaginally inseminated heifers (Table 16).

Analysis of variance showed that site of semen deposition was not a significant source of variation for percentages of any sperm trait in the cervix. The effect of cow nested within site of semen deposition was significant for proportions of live sperm, live normal sperm, pyriform head sperm, live pyriform head sperm, elongated head sperm, flat head sperm, live flat head sperm and cratered head sperm in the cervix. Sample location within the cervix significantly affected only proportions of live sperm, live normal sperm and live pyriform head sperm in the cervix. The interaction of semen deposition site with sample location was not a significant source of variation for percentages of any cervical sperm trait.

No significant difference existed between vaginal and intrauterine inseminated heifers for proportions of any sperm trait in samples from cervical quarters for the central or peripheral cervical regions (Appendix Tables 19, 20, 21). Similarly, there were no significant differences within cervical quarter between the central and peripheral cervical regions after vaginal or intrauterine insemination for percentages of any sperm trait. For specific sperm abnormalities, samples from the peripheral cervix in vaginally inseminated heifers revealed significantly greater percentages of live decapitated sperm in the most cranial quarter of the cervix than in the two most caudal quarters (Appendix Table 21). Otherwise, no significant differences existed among cervical quarters within central or peripheral cervical regions due to insemination site. There appeared, however, to be a distinct tendancy towards elevated levels of viable sperm in peripheral vs central cervical regions for either insemination site (Appendix Table 19).

When cervical data were pooled across sites of insemination, no significant differences existed in percentages of sperm traits among cervical quarters within central or peripheral cervical regions (Table 19); however, significantly greater proportions of live sperm and live

Table 19. Least squares means ± standard errors for percentages of sperm quality traits in the cervix by cervical region[a] and cervical quarter[b] and pooled across insemination sites. Experiment 2.

	Cervical Region							
Sperm Trait	Central				Peripheral			
	Qtr1	Qtr2	Qtr3	Qtr4	Qtr1	Qtr2	Qtr3	Qtr4
Live	38.3 ± 4.5*	45.1 ± 4.5	48.3 ± 4.5	52.0 ± 4.5	60.3 ± 4.5*	59.3 ± 4.5	55.5 ± 4.5	62.2 ± 4.5
Normal	73.5 ± 3.1	70.8 ± 3.1	72.4 ± 3.1	74.4 ± 3.1	70.0 ± 3.1	70.1 ± 3.1	73.6 ± 3.1	71.1 ± 3.1
Abnormal	26.6 ± 3.1	29.3 ± 3.1	27.6 ± 3.1	25.6 ± 3.1	30.0 ± 3.1	30.0 ± 3.1	26.4 ± 3.1	28.9 ± 3.1
Live Normal	26.7 ± 3.4*	29.7 ± 3.4	33.8 ± 3.4	37.5 ± 3.4	42.2 ± 3.4*	41.3 ± 3.4	40.8 ± 3.4	45.2 ± 3.4
Live Abnormal	11.7 ± 2.6	15.3 ± 2.6	14.5 ± 2.6	14.5 ± 2.6	18.1 ± 2.6	18.0 ± 2.6	14.7 ± 2.6	17.0 ± 2.6

[a]Cervical region: Central: obtained by curettage of cervical mucosa proximal to cervical canal
Peripheral: obtained by vacuum deeper between 1^{o} and 2^{o} folds of cervical mucosa

[b]Cervical Quarter: Qtr. numbered sequentially 1-4 from vaginal to uterine aspect

*Cervical region means within cervical quarter differ ($p<.05$), means so designated differ

normal sperm were found in the most caudal quarter of peripheral cervical regions than in the most caudal quarter of central cervical regions. No significant difference existed among cervical quarters within central or peripheral cervical regions or within quarters between central or peripheral cervical regions for proportions of other sperm traits.

When specific abnormalities were considered (Appendix Table 22), significant differences were found to exist only between proportions of cratered sperm in the two most cranial quarters in central cervical regions. For percentages of all other specific sperm abnormalities, no significant differences existed among cervical quarters within central or peripheral cervical regions or within quarters between central and peripheral cervical regions.

Table 20 summarizes cervical data pooled across semen deposition sites and cervical quarters. Samples from the peripheral cervix exhibited significantly greater proportions of live sperm and live normal sperm than did those from the central cervical region. In addition to percentages of normal sperm (Table 20), sperm having specific head abnormalities and sperm both alive and having specific head abnormalities (Appendix Table 22) were each not significantly different between central and peripheral cervical regions, with the exception of live pyriforms, which were greater in the peripheral cervix.

The cervical data from this experiment echo those from Experiment 1. The results indicate that a viability gradient existed between sperm found in the central vs peripheral aspects of the cervical canal. Live sperm and live normal sperm appeared to be preferentially located deeper between the folds of the cervical mucosa. Sperm in the other classifications, however, appeared more uniformly distributed across the cervical canal profile. These results indicate that sperm viability and, to a lesser extent, sperm morphology (i.e. the absence of abnormal morphology) were determinants of sperm location in the deep vs more central cervical mucosa.

Because few sperm were recovered in samples of vaginal mucus from each vaginally inseminated heifer, qualitative evaluation of that sperm population in those heifers was not possible. Only data from intrauterine insemination were obtained, and although comparison

Table 20. Least squares means ± standard errors for percentages of sperm traits in the cervix by cervical region and pooled across insemination sites and cervical quarters. Experiment 2.

Sperm Trait	Cervical Region	
	Central	Peripheral
Live	45.9 ± 2.4	59.3 ± 2.4*
Normal	72.8 ± 2.3	71.2 ± 2.3
Abnormal	27.2 ± 2.3	28.8 ± 2.3
Live Normal	31.9 ± 1.4	42.4 ± 1.4**
Live Abnormal	14.0 ± 1.9	17.0 ± 1.9

[a]Sample Location: Central: obtained by curettage of cervial mucosa proximal to cervical canal
Peripheral: obtained by vacuum deeper between 1^{o} and 2^{o} folds of cervical mucosa

*means within lines differ ($p<.05$)
**means within lines differ ($p<.01$)

of effects of vaginal insemination to intrauterine insemination was central to the experimental design, those vaginal mucus data are presented nonetheless (Table 21).

Analysis of variance showed that cows were a significant source of variation for proportions of pyriform head sperm, live pyriform head sperm, tapered head sperm, decapitated sperm, elongated head sperm, flat head sperm, live flat head sperm and live cratered head sperm in the vaginal mucus. The effect of sample location was significant for only percentages of live sperm and live normal sperm.

Proportions of sperm traits in the vaginal mucus did not differ from those in most other tract locations (Table 21). Percentages of live sperm and live normal sperm in the vaginal mucus were significantly lower than in the uterus, but did not differ from the inseminate or the cervix. Proportions of normal sperm, abnormal sperm, and live abnormal sperm did not differ between the vaginal mucus and the other sample locations or the inseminate. When specific head abnormalities were considered alone and in conjunction with their viability, no differences were evident between the vaginal mucus and the other sample locations or the inseminate for percentages of any of those sperm traits (Appendix Table 24).

These limited results suggest that sperm presence in the vaginal mucus after intrauterine deposition of low viability semen was related to sperm viability. The significantly reduced proportions of live sperm and live normal sperm in the vaginal mucus compared to the uterus suggests a preferential retention of those sperm, particularly in light of the lack of difference between uterine and vaginal mucus for percentages of live abnormals. It should be noted, however, that vaginal mucus levels of live abnormal sperm were nearly half those in the uterus suggesting a tendancy towards uterine retention of live abnormals, also (Table 21).

Analysis of variance showed that site of semen deposition was not a significant source of variation for percentages of any sperm trait in the retrograde mucus. The effect of cow nested within site of semen deposition had a significant effect on proportions of live sperm, live normal sperm, total live abnormal sperm, live primary abnormals, pyriform head sperm, live pyriform head sperm, tapered head sperm, live tapered head sperm, decapitated sperm,

Table 21. Least squares means ± standard errors for percentages of sperm quality traits in the vaginal mucus of intrauterine inseminated heifers compared to those in other sample locations. Experiment 2.

Sperm Trait	Vaginal Mucus	Inseminate	Cervix		Uterus
			Central	Peripheral	
Live	28.9 ± 6.3	32.1 ± 6.3	46.2 ± 6.3	55.8 ± 6.3	71.4 ± 6.3**
Normal	68.9 ± 7.8	71.2 ± 7.8	72.1 ± 7.8	70.4 ± 7.8	76.2 ± 7.8
Abnormal	31.1 ± 7.8	28.8 ± 7.8	27.9 ± 7.8	29.6 ± 7.8	23.8 ± 7.8
Live Normal	21.1 ± 4.6	23.3 ± 4.6	31.3 ± 4.6	39.2 ± 4.6	56.9 ± 4.6**
Live Abnormal	7.8 ± 3.2	8.8 ± 3.2	14.9 ± 3.2	16.7 ± 3.2	14.5 ± 3.2

**Differs from vaginal mucus mean ($p<.01$)

live decapitated sperm, elongated head sperm, flat head sperm, live flat head sperm and cratered head sperm in the retrograde mucus. Sample location was shown by analysis of variance to be a significant source of variation for percentages of live sperm, live normal sperm, live primary abnormals, total live abnormal sperm, tapered head sperm, live tapered head sperm, flat head sperm and cratered head sperm in the retrograde mucus. The interaction of semen deposition site with sample location had a significant effect on proportions of live sperm, live normal sperm, total live abnormal sperm, live primary abnormals, pyriform head sperm and live pyriform head sperm in the retrograde mucus.

Percentages of some sperm traits in the retrograde mucus differed with site of semen deposition. Significantly lower proportions of live sperm, live normal sperm and live abnormal sperm were found in the retrograde mucus from intrauterine-inseminated heifers than from vaginally inseminated heifers (Table 22). No significant differences existed between vaginal and intrauterine insemination for retrograde mucus levels of morphologically normal sperm or total abnormal sperm. This illustrates the wastage in terms of viable sperm that occurrs after vaginal semen deposition as opposed to intrauterine insemination and demonstrates the lack of selective sperm removal by the mucus based upon sperm morphology.

Significantly greater percentages of live sperm, live normal sperm and live abnormal sperm were present in the retrograde mucus than in the inseminate for vaginally inseminated heifers, but not for intrauterine inseminated heifers (Table 22). Intrauterine inseminated heifers exhibited significantly greater percentages of live sperm and live normal sperm in the peripheral cervix and uterus than in the retrograde mucus while vaginally inseminated heifers did not. When specific abnormalities were considered, no significant differences were found between sites of insemination for retrograde mucus levels of the abnormalities or between retrograde mucus levels and levels in the other sample locations with the exception of sperm having pyriform heads and live pyriform head sperm (Appendix Table 25 and Appendix Table 26, respectively), for which significantly lower percentages occurred in the retrograde mucus after vaginal vs intrauterine insemination. Significantly reduced proportions of sperm having pyriform heads were also noted in cervical and uterine samples than in the retrograde mucus

Table 22. Least squares means ± standard errors for percentages of sperm quality traits in the retrograde mucus sperm population compared to those in other sample locations, by insemination site.[a] Experiment 2.

Sperm Trait	Site	Retrograde Mucus	Inseminate	Cervix		Uterus
				Central	Peripheral	
Live	v	65.6 ± 5.0	32.1 ± 5.0**	45.6 ± 5.0	62.8 ± 5.0	83.6 ± 5.0
	u	24.2 ± 6.4^{++}	32.1 ± 5.0	46.2 ± 5.0	55.8 ± 5.0**	71.4 ± 5.0**
Normal	v	63.6 ± 6.9	71.2 ± 6.9	73.4 ± 6.9	72.1 ± 6.9	67.0 ± 6.9
	u	58.5 ± 8.7	71.2 ± 6.9	72.1 ± 6.9	70.4 ± 6.9	76.2 ± 6.9
Abnormal	v	36.4 ± 6.9	28.8 ± 6.9	26.6 ± 6.9	27.9 ± 6.9	33.0 ± 6.9
	u	41.5 ± 8.7	28.8 ± 6.9	27.9 ± 6.9	29.6 ± 6.9	23.8 ± 6.9
Live Normal	v	43.5 ± 3.8	23.3 ± 3.8*	32.5 ± 3.8	45.6 ± 3.8	56.1 ± 3.8
	u	18.3 ± 4.8^{++}	23.3 ± 3.8	31.4 ± 3.8	39.2 ± 3.8*	56.9 ± 3.8**
Live Abnormal	v	22.1 ± 2.9	8.8 ± 2.8*	13.1 ± 2.9	17.3 ± 2.9	27.5 ± 2.9
	u	5.9 ± 3.6^{+}	8.8 ± 2.9	14.9 ± 2.9	16.7 ± 2.9	14.5 ± 2.9

[a]Insemination site v = vaginal semen deposition
u = intrauterine semen deposition

*Differs from retrograde mucus mean ($p<.05$)

**Differs from retrograde mucus mean ($p<.01$)

$^{+}$Column means within sperm trait differ ($p<.05$)

$^{++}$Column means within sperm trait differ ($p<.01$)

of vaginally inseminated heifers (Appendix Table 25) while levels of sperm both alive and having pyriform heads were significantly reduced only in cervical samples from those heifers (Appendix Table 26). Proportions of decapitated sperm, while not significantly different between the retrograde mucus and the other tract locations or the inseminate for either insemination site, were notably comparable with inseminate levels of the trait and apparently much lower than in the other tract locations (Appendix Table 26). This indicates the removal of these abnormal forms from the female tract via the retrograde mucus after vaginal or intrauterine insemination. The combination of the low viability of the decapitated sperm in the inseminate (Appendix Table 26) with their inherent lack of motile ability appears to have doubly assured their removal.

These data show that the retrograde mucus sperm population in vaginally inseminated heifers were qualitatively quite similar to those in the other reproductive tract locations and contained greater levels of viable sperm than did the mucus from intrauterine inseminated heifers (Table 22). This suggests that retrograde mucus was a vehicle for removing viable sperm from the vaginally inseminated heifers. The lower levels of viable sperm in retrograde mucus from the intrauterine vs vaginal inseminated heifers indicates that viable sperm were retained in the tracts of the former heifers and nonviable sperm were removed via the mucus. The similarity between the two insemination sites for levels of sperm morphology traits (normal sperm and abnormal sperm) in the retrograde mucus coupled with the lack of differences between retrograde mucus and the other sample locations for those morphology traits suggest sperm presence in retrograde mucus, when based upon morphology alone, was random. Decapitated sperm were the exception; however, most were dead upon insemination. These results indicate that sperm presence in retrograde mucus was viability related for both vaginal and intrauterine insemination. In the former, a greater level of live sperm were present; in the latter, a greater level of dead sperm. Sperm morphology alone did not appear to play a role in sperm presence in retrograde mucus.

The results of *in vitro* incubation of inseminate samples are presented in Table 23. Chi-squared analysis showed that the change in level of live normal sperm and the change in

level of live abnormal sperm did not differ significantly. This result indicates, as in Experiment 1, that differential death of the normal vs abnormal sperm used in this experiment neither occurred *in vitro* nor, presumably *in vivo*.

Table 23. Least squares means ± standard errors for percentages of live normal sperm[a] and live abnormal sperm[b] in sample before and after *in vitro* incubation.[c] Experiment 2.

Sperm Trait	Incubation[c]		Change
	Before	After	
Live Normal	23.3 ± 2.7	24.0 ± 2.7	+0.7
Live Abnormal	8.8 ± 2.0	7.0 ± 2.0	-1.8

[a]Percentage of sperm both alive and having normal morphology
[b]Percentage of sperm both alive and having abnormal morphology
[c]Semen incubated 11.72 ± .13 hours to coincide with time of deposition and recovery of sperm in the tract

Discussion

Introduction

From the outset, the primary purpose of this investigation was to determine whether the bovine cervix functions as a selector of morphologically abnormal sperm, particularly those having abnormalities of the head. As presented in the review of literature, circumstantial evidence indicates that selection of sperm may occur as a result of passage through the cervix. In order to establish what occurs in the bovine cervix relative to the transport of morphologically normal vs abnormal sperm, the primary question to be answered would be whether any qualitative differences develop between the sperm populations of the inseminate and the uterus as a result of cervical passage of sperm to the uterus. Should differences become evident, the next logical step would be elucidation of the mechanisms involved. Comparison of the results of vaginal semen deposition to those of intrauterine semen deposition would indicate the cervical involvement in determining the uterine sperm population. Additional resolution of cervical involvement would be obtained by evaluation of any progressive change cervical sperm populations, Further, the evaluation of the sperm population in the mucus expelled by the cervix would indicate what types of sperm are removed via this route. Because both viability and morphology of sperm can clearly affect fertility, and

the transport of sperm in the female is related to the viability, and morphology of those sperm, the evaluation of the sperm in sperm transport studies must include both morphology and viability. This is important not only to eliminate potential confounding of morphology effects by viability effects but also to determine whether changes that may occur are due to differential viability as they may be related to sperm morphology.

Study 1 (Preliminary Studies)

The intent of Study 1 (preliminary studies) was simply to determine whether there were sufficient qualitative differences between the inseminate and uterine sperm populations in vaginally inseminated cows to warrant further investigation of cervical effects on transport of abnormal sperm. In addition, those studies permitted development of some techniques that were employed when further investigations were conducted.

Vital Staining of Fixed Sperm

The results of the two validation trials (Table 1, Table 2, Table 3, Figure 9) demonstrate that vital staining of bovine sperm preserved in Karnovsky's (1965) glutaraldehyde-paraformaldehyde fixative reproduced the results of a conventional method for vitally staining unfixed (air fixed) sperm when eosin-aniline blue vital stain (Shaffer and Almquist, 1948) was used regardless of method of killing (Trial 1, Table 2) or proportion of killed cells present (Trial 2, Table 3). This was true when half-stained sperm were counted as unstained (live) sperm (Table 1). Historically, half-stained sperm have been considered dead and counted with stained sperm (Lasley et al., 1942; Shaffer and Almquist, 1948; Blom, 1950). Progressive staining of the sperm head from its posterior to anterior aspect has been reported to occur in sperm that have recently lost their motile ability and to result in differences in the intensity of staining in the dried smear (Emik and Sidwell, 1947). Partial staining may indicate recent sperm death as a result of mechanical or chemical injury to the cell during the staining pro-

cedure (Emik and Sidwell, 1947; MacPherson, 1960) although this view is not universally held (Swanson and Bearden, 1951). The results of Trial 1 support the contention of the former authors, showing reduced levels of half-stained sperm in fixed vs unfixed samples of semen before exposure to lethal conditions, (Table 1) suggesting some protection against the effects of the staining procedure was afforded by the chemical fixation of sperm prior to staining. That the "protection" did not completely preclude staining was indicated by the level of stained sperm in fixed semen samples and the lack of significant differences between vital staining results of fixed and unfixed semen when half-stained sperm were counted with unstained sperm (Table 1). Those results indicate that the source of differences in vital staining between fixed and unfixed semen lay with the half-stained sperm in unfixed semen samples. Thus, fixation of sperm with glutaraldehyde- paraformaldehyde fixative (Karnovsky, 1965) prior to vital staining with eosin-aniline blue appears to have protected the sperm from some detrimental effect(s) inherent to the vital staining procedure itself, as indicated by reduced proportions of half-stained sperm in fixed semen before kill treatment (Table 1). An accurate picture of the sperm population's viability before (and not resulting from) the vital staining procedure is indicated by these results.

In Trial 2, half-stained sperm were counted with unstained sperm and the results echoed those of Trial 1, showing no differences in the outcome of eosin-aniline blue vital staining of fixed vs unfixed semen when known levels of killed sperm were added to unkilled sperm (Table 3, Figure 9). The means for unfixed vs fixed sperm did not differ significantly at any level of added killed sperm and the lines representing the means for fixed and unfixed sperm did not differ.

When taken together, the results of the two trials demonstrate that the eosin-aniline blue vital staining results for unfixed (air fixed) bovine semen are reproducible using the same semen preserved in Karnovsky's (1965) glutaraldehyde-paraformaldehyde fixative regardless of lethal treatment or the level of nonviable sperm present. The chemical fixation method employed in present work prior to vital staining reduced the proportion of half stained sperm by decreasing some artifact associated with the vital staining of chemically unfixed sperm.

This method may also be advantageous not only by decreasing the time pressure that could occur when the viability of numerous semen samples must be simultaneously determined, but can be performed using commonly available laboratory reagents and optics. In addition, it would appear to afford flexibility in sample utilization by allowing brightfield microscopic examination of vitally stained smears, phase contrast and differential interference contrast evaluation of wet mounts and primary fixation of the sample for electron microscope work using sperm from a sample of semen that had undergone only one chemical treatment. A higher standard error was associated with the chemical fixation method's vital staining results that is difficult to explain (Table 1, Table 2).

Since the work of Sabatini et al. (1963), aldehydes have been the fixatives of choice for electron microscopy because of their ability to: preserve cellular fine structure (Glauert, 1975), protect the continuity of membrane systems (Franzini-Armstrong and Porter, 1964; Tormey, 1964), and maintain cellular osmotic properties (Jard et al., 1966). The aldehydes have also been chosen for use as fixatives for spermatozoa and the qualitative evaluation of aldehyde fixed sperm is not without precedent. In wet smears, the morphology of glutaraldehyde-fixed sperm has been shown to compare favorably with that in wet smears of unfixed sperm in the bull (Johnson et al., 1976; Harasymowycz et al., 1976) and the boar (Pursel and Johnson, 1974). The results of vital staining of sperm preserved in formalin-based fixatives has been demonstrated to be comparable to those using unfixed sperm from the bull (Dott, 1975; Dott and Foster, 1975; Sekoni et al., 1981) boar, rabbit and stallion (Dott and Foster, 1975). An explanation as to why the vital staining of fixed vs unfixed cells was comparable was offered by Yip and Auersperg (1972) when they concluded that glutaraldehyde fixation preserved structural differences in the region of the cell membrane of damaged and undamaged cells in culture, which allowed reproduction of the results of vital staining in unfixed cells.

The dye exclusion test has been one of the more common methods of determining sperm viability as evidenced by the variety of procedures in use (e.g. Hackett and MacPherson, 1965), and the longevity of its use (e.g. Lasley et al., 1942). Because time (Lasley et al., 1942; Campbell et al., 1956) and temperature (Hancock, 1951; Bishop et al., 1954) can

adversely affect a semen sample, so, too, can they adversely affect reliability of vital staining results (Lasley et al., 1942; Hancock, 1951; Bishop et al., 1954; Campbell et al., 1956). Ideally, the assessment of a sperm sample's viability would be performed immediately upon collection under isothermic conditions, but field conditions and experimental time constraints can often be less than optimum for obtaining credible vital staining results. A method that would preserve the status of the cellular characteristic used as the criterion of viability judgement (e.g. membrane integrity) could improve convenience and increase the reliability of assessing sperm viability. Because vital staining is based upon the principle of exclusion of certain stains by viable cells (Hanks and Wallace, 1958), a method of preserving the integrity status of the sperm cell membrane at the time of sampling while retaining the vital staining characteristics of the cell treated by that method would make the determination of sperm viability both reliable and convenient. The vital staining procedure whose validation has been described here appears to be a method that meets those criteria.

Trial 3

The results of the sperm transport portion of Study 1 showed that a uterine sperm population containing enriched levels of live sperm as determined by vital staining and morphologically normal sperm, compared to the inseminate, resulted after vaginal deposition of semen (Table 4). These results lend support to those of previous workers who have proposed that the cervix functions as a reservoir from which viable sperm depart towards the uterus during the sustained phase of sperm transport (Mattner, 1963, 1968; Dobrowolski and Hafez, 1970) and as an organ whose secretion facilitates removal of nonviable sperm towards the vagina (Mattner and Braden, 1969a; Mattner, 1973).

It has been demonstrated that viable sperm are necessary for the penetration of and passage through cervical mucus *in vitro* (Moghissi, 1973; Mortimer et al., 1982) and *in vivo* (Mattner and Braden, 1969a; Mattner, 1973; Ragni et al., 1985). In the present work, semen was deposited in the vaginae of cows and the appearance of sperm in their uteri implied cervical passage prior to arrival in the uterus. Thus, it would appear that the cervix was re-

sponsible for the enriched uterine population of viable sperm barring the transient effects of rapid sperm transport (Mattner, 1963a; Overstreet and Cooper, 1978). If the cervix does function as a barrier to nonviable sperm, as implied by these results, then uterine sperm numbers would be inversely related to the number of nonviable sperm deposited in the vagina. Increased levels of nonviable sperm inseminated could, therefore, result in decreased fertility when a lower than "threshold" (Elliott, 1978) number of viable uterine sperm resulted from cervical passage.

The enhanced proportion of morphologically normal sperm observed in the uterine vs inseminate population in this study suggests that the cervix may have been involved in the exclusion of abnormal sperm from the uterus. While the differences were not significant in each of the individual times post-insemination, possibly due to the low number of cows within each time subclass, the tendency towards elevated uterine proportions of normal sperm in each time subclass (Table 4) was borne out when data were pooled across times post-insemination which revealed a significantly greater proportion of normal sperm recovered in the uterine flush (Table 5). Although percentages of most specific sperm abnormalities tended to be reduced, but not significantly so, in the uterine vs inseminate sperm populations, percentages of sperm having coiled tails or abnormal acrosomes were significantly lower in the uterine population (Table 6). The *in vitro* ability of sperm to migrate through various media (Parrish and Foote, 1987) including mucus (Roslanowski and Koefoed-Johnsen, 1971; Koefoed-Johnsen, 1972) has been tied to their motility, which in the case of coiled tails is undoubtedly impaired. Failure to ascend the reproductive tract has also been attributed to impaired sperm motility *in vivo* (Mortimer, 1977; Insler et al., 1980; Ragni et al., 1985; Gonzales and Jezequel, 1985). It appears then, that the cervix may present a barrier to sperm having impaired motility; however, the low levels of sperm in the preliminary studies that possessed tail abnormalities which would impair motility makes it difficult to form definite conclusions on that basis.

Similar difficulties are encountered when drawing conclusions concerning the significantly reduced percentages of uterine sperm having abnormal acrosomes. The very low

levels of this abnormality (Table 6) interfere with meaningful interpretation of this result which, none the less, appears to warrant further study. This is undrescored by the report of Mitchell et al. (1985) who noted a reduction in the proportion of sperm possessing abnormal acrosomes recovered from the bovine uterus, cervix and vagina, as compared to that of the inseminate after intrauterine semen deposition. Those authors suggested that reduced motility of sperm having head abnormalities may have adversely affected retention of these sperm in the female tract. It has been demonstrated *in vitro* that sperm having abnormal head shape suffer a motility disadvantage compared to normal sperm, in terms of swimming speed, flagellar beat frequency and linearity of movement (Katz et al., 1981).

It is quite possible that the apparent reductions in levels of abnormal uterine sperm demonstrated by these preliminary studies were due to impaired motility of those sperm. The results show that the uterine sperm population after vaginal semen deposition contained enriched levels of live and normal sperm compared to the inseminate, indicating the removal of dead and abnormal sperm. While the cervix may have been involved in that process, it is equally possible that the reductions in abnormal sperm, at least in part, may have resulted from reduced survivability of the abnormals, preferential phagocytosis of abnormal sperm by leukocytes or selective elimination of abnormals by retrograde removal from the cervix and uterus to the vagina. The results strongly indicated, in any event, that more detailed investigations on mechanisms of sperm selection were warranted.

Study 2

Results of Study 1 indicated that the cervix may have been involved in limiting the presence of dead sperm and morphologically abnormal sperm in the uterus after vaginal semen deposition. Further experiments were conducted to more critically define the effect of the cervix on the quality of the uterine sperm population and to differentiate the effects of sperm selection for viability from those for sperm morphology.

Uterine Sperm

The results of Experiment 1 and Experiment 2 indicate that the site of semen deposition had little effect on sperm quality in the uterus 12 hours after insemination (Table 7 and Table 16, respectively). In Experiment 1, where highly viable semen was used (i.e. 91% intact acrosomes) and sperm abnormalities included subtle along with the gross forms (Figure 5), no significant differences existed between the uterine sperm populations of vaginal vs intrauterine inseminated heifers when sperm viability, sperm morphology or the viability of the morphological types were considered (Table 7, Appendix Table 3, Appendix Table 4). There also were no significant differences between inseminate and uterine sperm populations for viability, morphology or the viability of the morphological types after deposition of semen in either location. When semen of low viability was used (i.e. 32% intact acrosomes) and only the grossly abnormal sperm were counted as abnormal (Experiment 2), uterine sperm quality between the two insemination sites again did not differ significantly for most of the sperm quality traits in the uterus other than proportions of live abnormal sperm (Table 16, Table 17, Appendix Table 17). As in Experiment 1, the inseminate and uterine sperm populations for either insemination site did not differ when only the morphology of sperm was considered (Table 16, Table 17). Unlike Experiment 1, greater proportions of live sperm, live normal sperm and live abnormal sperm were present in the uterus than in the inseminate for both insemination sites. It should be noted that overall, uterine sperm viability (regardless of morphology) tended to be greater after vaginal insemination as compared to intrauterine insemination. This was much more apparent when low viability semen was used (Experiment 2, Table 16) than with high viability semen (Experiment 1, Table 7). This is not unexpected, considering that viability effects would be most pronounced when low viability semen was used. In a similar vein, abnormal uterine sperm were subtly lower after uterine vs vaginal deposition while uterine levels of normal sperm were subtly enriched, suggesting a uterine effect on the morphology of the uterine sperm population. These trends suggest that sperm viability may have greater importance than morphology in sperm gaining access to the uterus

or remaining in the uterus, particularly after vaginal semen deposition but also after intrauterine insemination.

The comparison of uterine sperm populations 12 hours after vaginal and intrauterine insemination in both Experiment 1 and Experiment 2 indicates that uterine sperm quality was primarily determined independent of the site of semen deposition (Table 7, Table 16). The comparison of inseminate to uterine sperm quality in Experiment 2 demonstrates that differences did exist between the two sperm populations, which were obviously related to sperm viability when low viability semen was used (Table 16), but for the most part were not affected by site of semen deposition. Had an effect of semen deposition site occurred, differences should have been apparent not only for uterine sperm quality traits between the two insemination sites but also in terms of sperm quality of the inseminate vs the uterine populations for each site. The disparity between the two experiments for levels of viable sperm (91% and 32% intact acrosomes, Table 7 and Table 16) should have been large enough for any viability effects relative to semen deposition site to become apparent. Similarly, proportions of abnormal sperm inseminated in each experiment (44% and 28%; Table 7, Table 16) should have been sufficient to elicit a morphology effect. A semen deposition site effect is indicated by the greater uterine levels of live abnormal sperm after vaginal semen deposition than after intrauterine deposition in Experiment 2 (Table 16) and the tendency towards enhanced uterine sperm viability in both experiments (Table 7, Table 16). However, the enhanced uterine levels of live abnormal sperm after vaginal insemination may have resulted from the greatly reduced percentage of decapitated sperm in the uterus after vaginal insemination (Table 17) of predominately dead decapitated sperm (Appendix Table 17). This suggests that vaginal semen deposition results in more efficient removal of sperm based upon viability than does intrauterine insemination. On the other hand, the subtle differences between the two insemination sites in each experiment for levels of sperm morphology imply that intrauterine semen deposition may result in a morphologically "cleaner" uterine sperm population. It appears, then, that uterine sperm viability (regardless of morphology) and uterine sperm morphology were in the most part, only subtly affected by the site of

insemination. In practical terms, these results indicate that sperm viability was a major determinant of sperm presence in the uterus regardless of site of semen deposition. The results also suggest that intrauterine semen deposition, as practiced in artificial insemination, is not detrimental to uterine sperm quality in terms of reduced levels of live abnormals, when compared to vaginal deposition as occurs in natural service.

When data were pooled across insemination sites in both Experiment 1 and Experiment 2, the results indicated that differences between the inseminate and uterine sperm quality were related predominantly to sperm viability (Experiment 2) and, to a lesser degree, to sperm morphology (Experiment 1). Most important in Experiment 1 were the significantly greater levels of normal sperm and significantly lower levels of abnormal sperm (total abnormals and primary abnormals) in the uterus as compared to the inseminate (Table 8). However, live normal sperm did not differ between the inseminate and uterine populations while live abnormals were significantly decreased and live primary abnormals approached significant reduction ($.05 < p < .10$) in the uterus. The nonsignificant (though apparent) decrease in levels of total live sperm in the uterus vs the inseminate appeared to have offset the increased uterine levels of normal sperm such that when the viability of the normal sperm was considered, the uterine population of live normal sperm did not differ from that in the inseminate. The relationship between levels of abnormal sperm (total abnormals and primary abnormals) in the uterus vs the inseminate did not appear to be as affected by the downward shift in total live sperm between the inseminate and uterus as the normal sperm were. This resulted in a very similar relationship between inseminate and uterine populations for levels of live abnormals and live primary abnormals as there was for total abnormals and primary abnormals when only morphology was considered (i.e. reduced uterine levels). These results indicate that a sperm quality shift occurred between insemination and recovery of sperm from the uterus based upon their morphology which resulted in reduced uterine levels of abnormal sperm compared to the inseminate.

The use of low viability semen (Experiment 2) resulted in no differences between the inseminate and uterine sperm populations when only sperm morphology was considered

(Table 16, 17). When the viability of the morphological types was examined, however, significantly greater levels of viable sperm were found in the uterine population regardless of morphology (Table 17). This strongly indicated that differences between the inseminate and uterine sperm populations in Experiment 2 were driven by sperm viability. However, of the abnormal sperm in the inseminate (28.8% of total sperm, Table 16) nearly half were dead decapitated sperm (11.5% of total sperm; difference between total decapitated and live decapitated, Appendix Table 18) when live abnormal sperm (predominately primary abnormals) constituted only 8.8% of total sperm inseminated. Therefore the significantly greater levels of live abnormal sperm in the uterus vs the inseminate (Table 16) may have been due to the inability of decapitated sperm to cross the cervix (due to their lack of flagellar abilities), which offers an explanation for the significant insemination site differences between vaginal and intrauterine semen deposition for live abnormals (Table 16).

These results indicate that presence of sperm in the uterus was primarily dependant upon their viability; however, with high sperm viability (Experiment 1), the effects of sperm morphology also became apparent (Table 8). The effect of sperm morphology *per se* on sperm presence in the uterus should have been most evident when the effects of viability were minimized (i.e. when highly viable semen was used), and least evident with the use of low viability semen. The present results show no viability effects and subtle morphology effects on the presence of sperm in the uterus when highly viable semen was used (Experiment 1). Very obvious viability effects and no apparent morphology effects resulted when semen of low viability was employed (Experiment 2). In these experiments the effects of sperm morphology on their presence in the uterus was either considerably subordinate to the effects of sperm viability or not completely detectable by the methods employed.

Experiments 1 and 2 were designed such that intrauterine and vaginal insemination of heifer pairs would isolate cervical effects from confounding uterine effects. The finding that semen deposition site had no effect on uterine sperm quality in the present experiments could mistakenly be interpreted as indicating no involvement of the cervix in determining uterine sperm quality. That interpretation would be accurate if sperm transport in the female was not

the dynamic process characterized by progressive cranial movement of viable sperm and the progressive caudal movement of sperm being removed by retrograde flow. *In vitro* it has been demonstrated that viable sperm can penetrate cervical mucus while nonviable sperm cannot (Moghissi, 1964; Gaddum-Rosse et al., 1980a,b). *In vivo*, after vaginal semen deposition, nonviable or immotile sperm do not gain access to the cervix or are removed from the cervix (Mattner and Braden, 1969a; Lightfoot and Restall, 1971) presumably by retrograde mucus flow towards the vagina. Thus, the cervix has been shown to be a barrier to the entry of nonviable sperm to the female tract and provides a vehicle for their removal should death occur after their gaining access to the tract. Viable sperm enter the cervix from the vagina via the mucus (Tampion and Gibbons, 1962a; Mattner, 1966) and migrate through the mucus between the folds (Lightfoot and Restall, 1971), grooves (Mullins and Saacke, 1989) or cervical villi (Mattner, 1969a) of the cervical mucosa towards the uterus. The concept of sperm transport through the cervix illustrates the role of that organ relative to the viability of sperm en route to the uterus, where viable sperm are allowed to enter or to be retained while nonviable sperm are excluded from entry or are removed. Likewise, nonviable uterine sperm are removed towards the vagina in the retrograde mucus flow (Mattner, 1963b, Lightfoot and Restall, 1971). Viable sperm, on the other hand, disperse randomly into the cervix and uterus after their introduction into the uterus (Mattner, 1963b; Mattner et al., 1969). Those viable sperm that disperse toward the cervix may colonize the cervical mucosa (Mattner, 1963b; Mattner et al., 1969; Lightfoot and Restall, 1971) and possibly reenter the uterus (Larsson and Larsson, 1985; Larsson, 1988). In view of the literature, it would be inaccurate to interpret the results of the present studies as indicative of noninvolvement of the cervix in determining the viability of the uterine sperm population.

On the contrary, there is strong evidence that supports the role of the cervix as a barrier to immotile or dead sperm. There is also evidence in the literature that indicates that the cervix may function to determine the morphology of the sperm that pass across it. Bergman (1955) related the ability of human sperm to cross the cervix and enter the uterus to their morphology and motility, noting that the cervix appeared to retard the progress of ab-

normal sperm and thus acted as a selector against abnormal sperm. Mortimer et al. (1982) on the other hand, concluded that abnormal human sperm effected their own selection due to midpiece and tail defects that impaired motility. Differential motility of normal vs abnormal sperm has also been associated with reductions in female tract levels of abnormal sperm in the rabbit (Mortimer, 1977) and mouse (de Boer et al., 1976). A physiological deficiency (Nestor and Handel, 1984) or reduced survival (Mortimer, 1977) of abnormal sperm have also been presented as possible reasons for reduced levels of abnormal sperm recovered from the female genital tract. In the bovine, Mitchell et al. (1985) and Larsson (1988) reported reduced uterine levels of abnormal sperm after intrauterine semen deposition. The former authors suggested that impaired motility of the abnormal sperm may have affected retention of those sperm in the female tract while the latter authors suggested impaired motility, and/or a selective removal mechanism resulted in preferential elimination of abnormals. Although there was evidence in both of those studies that abnormalities of the sperm tail were reduced compared to the inseminate, in neither case was a viability determination performed on sperm recovered from the tracts, making a judgement concerning sperm removal based upon motility an inferential one. In Experiment 1, abnormalities of the tail were very low (Table 8), thus eliminating that effect from consideration as a possible cause for the reduced uterine levels of abnormal sperm. Similarly, in Experiment 2 the levels of tail abnormalities *per se* were extremely low, to the extent that when included with levels of primary abnormals the percentage of total abnormals were in most cases identical to that for primary abnormals. It must be considered, however, that levels of decapitated sperm inseminated, while very low in Experiment 1 (1.6%, Appendix Table 3), were substantial in Experiment 2 (12.3%, Table 17). The relatively high levels of these sperm in Experiment 2 may have had an impact on the reduction of live abnormals (Table 18) since the majority of the decapitated sperm were dead upon insemination (0.8% live, Appendix Table 17 vs 12.3% inseminated, Table 17). However, the fact that decapitated sperm have no motile abilities doubly insured the exclusion of these sperm and underscores the importance of motility for sperm presence in the female tract.

It is difficult to adequately define the role of the cervix in the dynamic process that occurs in the female reproductive tract after the deposition of sperm. The results of the uterine aspect of these studies indicate that at 12 hours after semen deposition there was no evidence of cervical involvement in determining the quality of the uterine sperm population. This should not be interpreted as absolute proof that the cervix was not involved, just that the evidence was either not available or not discernible. The results of these studies do show that sperm presence in the uterus was based upon the morphology (Experiment 1) and the viability (Experiment 2) of the sperm, with sperm viability having the overriding effect.

Cervical Sperm

The results of Experiment 1 and Experiment 2 demonstrate the existence of a sperm population composed of greater levels of viable sperm within the folds of the cervical mucosa than at the more central aspects of the cervical canal (Table 9, Table 20). The greater level of live normal sperm in this area imply some advantage of those sperm over the other types for habitation of the mucosal folds not indicated by previous workers. Other sperm types showed relatively uniform distribution by type throughout the cervix.

The greater levels of live sperm and live normal sperm between the folds of the cervical mucosa (as compared to the more central cervix) in the present experiment are in agreement with the results of other authors (Mattner, 1966, 1968; Mattner and Braden, 1969b; Lightfoot and Restall, 1971) who reported a higher viability sperm population within the recesses of the cervical mucosa, indicating the maintenance of a viable population of sperm within that cervical region. However, there is no evidence in the literature of differences between the deeper and more central aspects of the cervical canal relative to the morphology of the sperm found there. The present findings, particularly those of Experiment 2 (Table 19) for live sperm (regardless of morphology), also lend support to the proposal that the mucosal folds constitute paths of least resistance (Mullins and Saacke, 1982, 1989) along which viable sperm migrate towards the uterus. Other sperm types showed uniform distribution throughout the cervix, which could be interpreted as evidence supporting the possible selection of sperm

within the cervix, and suggestion of selection for live normal sperm, not against other specific morphological types.

Considering the classic concept of sperm gaining entry to and being removed from the cervix, if selection were to occur in the cervix after vaginal semen deposition based upon sperm viability or morphology, a progressively more viable and/or morphologically normal sperm population would be expected in each successively more cranial quarter of the cervix. This would be expected whether the sperm were removed by retrograde mucus flow or simply impeded in their forward progress through the cervix. This was not apparent in the present experiments when the data were analyzed by insemination site (Appendix Tables 6,7,8 and Appendix Tables 19,20,21) or across insemination sites (Appendix Tables 9,10 and Appendix Table 22). In addition, no differences existed between the two insemination sites for levels of the sperm traits within cervical quarters, indicating that bypassing the cervix with artificial insemination did not eliminate an important barrier to undesirable sperm.

Mattner (1968, 1973) described retrograde removal in cervical mucus as a process that directed material of uterine origin to the more central aspect of the cervical canal presumably due to the alignment of mucus macromolecules and the greater rate of mucus flow in that area. It has been demonstrated that a considerable number of uterine-deposited sperm are lost to the vagina via this route (Suga and Higaki, 1971; Larsson, 1984; Larsson and Larsson, 1985; Mitchell et al., 1985) and some evidence of colonization of the cervical mucosa by these sperm has been reported (Mattner, 1963b; Mattner et al., 1969; Larsson and Larsson, 1985). Such a colonization process might be expected to result in greater levels of viable sperm existing in the cranial vs caudal portions of the cervix in samples from both the central and peripheral cervix after intrauterine insemination. In the present results, although no differences between central and peripheral samples within cervical quarters were observed in the intrauterine inseminated heifers, neither were there differences among cervical quarters within the central or peripheral samples for levels of the sperm traits. There was, however, a trend towards an increasingly more viable sperm population from caudal to cranial cervix after intrauterine insemination (Appendix Table 6, Appendix Table 19). This suggests a rela-

tively uniform colonization along the length of the cervix by viable sperm of uterine origin either from the sperm population presumably contained in the caudally flowing mucus of the cervical canal or possibly from direct entry into the cervical folds and grooves by uterine sperm at the level of the internal cervical os. The higher viability of the peripheral vs central regions, however, indicate selective colonization of the peripheral areas by viable normal sperm. These results, coupled with the lack of differences between sites of semen deposition for levels of the cervical sperm viability and morphology traits suggest that sperm selection may not have occurred within the cervix. They could also indicate that at the time of sampling (12 hours post-insemination) the cervical contents, evidence of selection had disappeared, unless the greater levels of live normal sperm in the peripheral vs central regions constitutes evidence of selection or its remainder.

Further investigations into cervical levels of sperm viability and morphology traits at different times post-insemination would further clarify what role, if any, the cervix plays in determining viability and morphology of the uterine sperm population.

Vaginal Mucus

Unfortunately, the vaginal mucus results for this study were incomplete due to the recovery of too few sperm from the vaginal mucus after vaginal deposition of low viability semen (Experiment 2) to qualitatively evaluate the populations. As a result, the effects of high and low viability semen on sperm quality in vaginal mucus could not be contrasted. Regardless of deficiencies in the data, the existing results show that site of insemination had no effect on vaginal mucus sperm quality (Experiment 1) and inicate that sperm presence in the vaginal mucus was related to their viability (Experiment 1 and Experiment 2).

The results of Experiment 1 show that there were no differences between the two sites of semen deposition for levels of any sperm trait in the vaginal mucus (Table 10, Appendix Table 12, Appendix Table 13) demonstrating that the site of semen deposition had no effect on levels of sperm traits in the vaginal mucus. The results show that sperm populations in the vaginal mucus and uterus were quite similar for both insemination sites when high viability

semen was used (Experiment 1, Table 10). Although levels of live sperm were significantly reduced in the vaginal mucus vs the inseminate for both insemination sites (Table 10), the levels of the other sperm traits in the vaginal mucus did not differ from those in the inseminate or from those in other locations in the tract regardless of insemination site. When low viability semen was used (Experiment 2), and data were only available from intrauterine inseminated heifers, reduced levels of live sperm and live normal sperm were evident in the vaginal mucus vs the inseminate while no differences existed between the vaginal mucus and the other tract locations or the inseminate for levels of those sperm traits (Table 21). In addition, levels of sperm traits in all other tract locations were not different from those in the vaginal mucus.

The similarity of insemination sites' effects on levels of vaginal mucus sperm traits suggests, based upon the limited data, that the cervix played a negligible role in changing the quality of the sperm population passing through it. If a sperm selection mechanism had been operating in the cervix, then levels of sperm traits in the vaginal mucus would be expected to differ between the two insemination sites, showing elevated vaginal mucus levels of sperm that had been selected out when compared to the other tract locations and the inseminate. However, the time of sampling may have been too late in relation to insemination for the effects of selection to be apparent in the vaginal mucus, due to mucus expulsion to the exterior.

The results of both Experiment 1 and Experiment 2 indicate that the presence of sperm in the vaginal mucus was related to their viability and not their morphology. In Experiment 1, when sperm were evaluated on morphology alone, levels of sperm traits in the vaginal mucus did not differ from those in the other tract locations or the inseminate, indicating non-discrimination against sperm based upon morphology (Table 10, Table 11). When viability was accounted for and data pooled across insemination sites, levels of total live sperm and live normal sperm in the vaginal mucus were lower than those in the inseminate, peripheral cervix and uterus, but not different from those in the central cervix, suggesting the latter location as their immediate site of origin (Table 11). However, while levels of live abnormals (total live abnormals and live primary abnormals) did not differ between the vaginal mucus and the other tract locations, they were reduced compared to the inseminate. This result suggests

that live abnormals were uniformly distributed throughout the more posterior regions of the tract. The reduced levels of live sperm (live, live normals, live abnormals and live primary abnormals) in the vaginal mucus compared to the inseminate indicate the presence of sperm in the vaginal mucus was based upon their viability, particularly in light of the high viability of the sperm inseminated. The results of Experiment 2 (Table 21), where only data from intrauterine inseminated heifers was available, were quite similar to those of intrauterine inseminated heifers in Experiment 1 (Table 10). When sperm were evaluated based upon their morphology alone, no significant differences existed between vaginal mucus and other tract locations or the inseminate or levels of normal sperm, total abnormal sperm or (Table 21). When sperm viability was considered, the vaginal mucus differed only from the uterine population for levels of live sperm and live normal sperm, while levels of live abnormals did not differ from those in any tract location or the inseminate. As with Experiment 1, these results indicate that sperm presence in the vaginal mucus was related to their viability. It is notable that a progressive increase in sperm viability occurred in samples as their locations approached the uterus.

These vaginal mucus results show that the site of semen deposition had no effect on the sperm quality of the vaginal mucus population and indicate that sperm presence in the vaginal mucus related to their viability and not their morphology. This agrees with the results of Mattner and Braden (1969a) and Lightfoot and Restall (1971) which demonstrated removal of nonmotile sperm to posterior portions of the tract by cervical mucus, and reinforces the importance of sperm viability to sperm retention within the female tract. These results do not support the proposal that a selection mechanism functions in the cervix against abnormal sperm by demonstrating the lack of insemination site effect in Experiment 1 and by showing the similarity of results after intrauterine insemination when semen of low or high viability was used. Alternatively, evidence of selection may have been present in the vaginal mucus at a time prior to sampling but may have disappeared by the time of sampling.

Retrograde Mucus

The results of Experiment 1 and Experiment 2, when taken together, reveal that the sperm population in retrograde mucus is of low viability after intrauterine insemination and is highly viable after vaginal semen deposition. The use of high viability semen (Experiment 1) resulted in reduced levels of total live sperm (Table 13) and live tapered head sperm (Appendix Table 14) in the retrograde mucus of intrauterine inseminated heifers as compared to vaginally inseminated heifers. When low viability semen was used (Experiment 2), reduced levels of total live sperm, live normal sperm and live abnormals were found in the retrograde mucus of the intrauterine vs vaginally inseminated heifers (Table 22). These results suggest that viable sperm were preferentially retained after intrauterine insemination as compared to vaginal insemination and demonstrate that sperm presence in the retrograde mucus was viability related.

The levels of sperm morphology traits in the retrograde mucus, the inseminate and the uterus were quite similar (Table 13, Table 22) regardless of insemination site and level of viable sperm inseminated, indicating the effects of sperm viability and not morphology in determining the retrograde mucus sperm population. The effect of morphology on sperm presence in the retrograde mucus was not masked by the effects of viability as evidenced by the similarity of results when semen of high and low viability (Experiment 1 and Experiment 2, respectively) was inseminated and only sperm morphology was considered. Had a morphology effect occurred, differences between the retrograde mucus levels of abnormal sperm and those in other samples would have been most evident when viability effects were minimized, i.e. when high viability semen was used (Experiment 1). Such an effect would have been expected to result in greater levels of abnormal sperm in the retrograde mucus than in the inseminate and, possibly, other tract locations. The lack of differences in levels of abnormal sperm between the retrograde mucus and the other samples is evidence against sperm morphology determining sperm presence in the retrograde mucus (Table 13, Table 14). This result is supported by those of Mitchell et al. (1985) who found that levels of sperm abnormalities, particularly primary and tertiary abnormalities, were generally uniform across

the inseminate, vagina, cervix and uterus 12 hours after intrauterine insemination. Larsson (1988), on the other hand, reported a reduced frequency of sperm having abnormal heads in the cervix and uterus compared to the inseminate 2 hours after intrauterine insemination. In neither of those studies was a viability determination performed on recovered sperm, making a conclusion concerning the basis for sperm distribution in the tract difficult to formulate, in light of the present evidence. In the present studies, it should be noted that, in Experiment 2, relatively high levels of decapitated sperm were inseminated and were present in the retrograde mucus, with obviously, but not significantly, lower levels present in the cervix and uterus regardless of insemination site (appendix Table 25). The presence of high proportions of this abnormality in the retrograde mucus relative to the other tract locations and of levels comparable to the inseminate indicate exclusion of that sperm trait from the female tract. Although the viability of this abnormality was low in the inseminate and in all tract locations (Appendix Table 24), the apparent exclusion of this abnormality should not be ascribed to low viability, but to impared motility, since decapitated sperm have no flagellar ability. This result underscores the importance of motility for sperm persistence in the female tract. In the present case, this sperm abnormality was doubly assured of exclusion, based upon its viability and its motile ability.

In Experiment 1, lower levels of live sperm (Table 13) and live tapered head sperm (Appendix Table 14) were present in the retrograde mucus vs the inseminate after intrauterine insemination. After vaginal insemination in that experiment, no differences existed between the retrograde mucus and the inseminate for levels of any sperm trait (Table 13, Appendix Table 14, 15). In Experiment 2, on the other hand, greater levels of total live sperm, live normal sperm and live abnormals were found in the retrograde mucus vs the inseminate of vaginally inseminated heifers (Table 22), while after intrauterine insemination, no differences existed between the retrograde mucus and the inseminate for levels of any sperm trait. These results suggest that retrograde mucus removed primarily dead sperm after intrauterine insemination, as evidenced by the reduced viability of the retrograde mucus sperm population vs the inseminate when either high or low viability semen (Experiment 1 and Experiment 2,

respectively) was used. These results also indicate that retrograde mucus contains mostly viable sperm after vaginal insemination as evidenced by the greater viability of the retrograde mucus sperm population vs the inseminate when low viability semen was used and the similarity between those populations' viability after deposition of high viability semen.

These contradictory roles could be explained by Mattner's (1968, 1973) model for the removal of uterine material via the cervix and the concept for the ingress of sperm from the vagina to the cervix (Mattner, 1966; Mullins and Saacke, 1989). In the former model, the material from the uterus is passively directed posteriorly through the more central aspects of the cervical canal by mucus flow towards the vagina. The latter concept describes the penetration of mucus by viable sperm (Roark and Herman, 1950; Moghissi et al., 1964; Goddum-Rosse, 1980a,b) which then migrate along mucus lines of strain (Tampion and Gibbons, 1972a; Mattner, 1966) or within sialomucin-rich channels laminated between layers of neutral mucin (Mullins and Saacke, 1989) to situate themselves in close proximity to the cervical mucosa (Mattner, 1966) within the folds (Lightfoot and Restall, 1971) or grooves (Mullins and Saacke, 1989) of the cervix. In the present results, the greater viability of the sperm population within the retrograde mucus vs the inseminate after vaginal insemination with low viability semen (Experiment 2, Table 22) best illustrates that viable sperm presumably penetrate mucus to a greater degree than nonviable sperm and, by utilizing the mucus as a means for gaining access to the cervix, appear more vulnerable to retrograde removal in mucus than are their nonviable counterparts. The fate of nonviable sperm is not clearly indicated by the present results. The similarity between the viability of the retrograde mucus sperm population and that in the inseminate when high viability semen was vaginally deposited (Experiment 1) offers further evidence of removal of viable sperm by retrograde mucus (Table 13).

After intrauterine semen deposition, nonviable sperm are removed in the retrograde mucus (Mattner, 1963b; Lightfoot and Restall, 1971) and viable sperm disperse randomly into the cervix and uterus (Mattner, 1963b; Mattner et al., 1969). Colonization of the cervical mucosa by presumably viable sperm after intrauterine semen deposition has been reported (Mattner, 1963b; Mattner et al., 1969; Lightfoot and Restall, 1971) and reentry of those sperm

to the uterus has been proposed (Larsson and Larsson, 1985; Larsson, 1988). Thus, when sperm are deposited within the uterus, they must either resist retrograde flow to avoid removal via the mucus or, having been caught in posteriorly flowing mucus, must penetrate it and migrate either back to the uterus or towards the deep cervical mucosa to escape retrograde removal. Comparison of the sperm population in the retrograde mucus to that in the inseminate after intrauterine semen deposition in the present study suggests that one or both of those events may have occurred. When high viability semen was used (Experiment 1) lower levels of viable sperm resulted in the retrograde mucus population compared to the inseminate and uterus after intrauterine insemination (Table 13). The intrauterine deposition of low viability semen (Experiment 2) resulted in no differences between the retrograde mucus and the inseminate for levels of viable sperm, but lower levels of viable sperm in the retrograde mucus compared to the uterus (Table 22). The results of both experiments indicate that after intrauterine insemination, nonviable sperm were removed via the retrograde mucus and viable sperm were retained within the uterus and provide further evidence that sperm presence in the retrograde mucus was dictated by sperm viability.

These retrograde mucus results demonstrate that the site of semen deposition affected the viability of the retrograde mucus sperm population by showing that a reduced viability sperm population resulted in the retrograde mucus after intrauterine insemination and an enhanced viability sperm population resulted after vaginal insemination. These results are consistent with the models for the ingress of vaginally deposited sperm to the uterus and the retrograde removal of material from the uterus. They also help to illustrate the importance of sperm viability and the insignificance of sperm morphology on sperm persistence in the female tract.

EYC Incubation

The results of the *in vitro* incubation of inseminate samples in egg yolk-sodium citrate extender showed that there was no difference between the change in viability of morphologically normal sperm and that of abnormal sperm in the semen used in either ex-

periment (Table 15, Table 23). This indicates that differential death of abnormal vs normal sperm did not occur as a result of *in vitro* incubation and suggests that differential death of abnormal vs normal sperm was not a factor that affected the transport of sperm in this study. These results agree with those of Mortimer (1977), who compared the relative survival of diploid (giant head) to haploid (presumably normal morphology) rabbit sperm incubated in three different media at 37°C for 7 hours. He found comparable survival between the two sperm types in each medium and concluded that although the proportion of dead diploid sperm were nearly three times that of dead haploid sperm at ejaculation, the two types appeared to be equally viable after ejaculation.

A possible reason for reduced retention of abnormal vs normal sperm in the female tract could be reduced survival of abnormal sperm. Nestor and Handel (1984) suggested "physiological disadvantage" as a possible explanation for the reduced numbers of structurally abnormal sperm they observed to reach the mouse oviduct. While these authors did not elaborate on the meaning of 'physiological disadvantage', differential survivability could be considered as a possible physiological disadvantage to the transport of abnormal sperm. Differential survival based on sperm morphology was not indicated by the results of the *in vitro* incubation and, although the incubation medium employed can not be claimed to duplicate the media provided by the female tract, the results suggest that it neither prevented nor promoted sperm death.

Summary

Two studies were conducted to determine the role of the bovine cervix in the filtration of morphologically abnormal sperm, particularly those having abnormal heads. In Study 1, 5 ml semen containing high levels of morphologically abnormal sperm was deposited in the vaginae of 12 Holstein cows and, after slaughter, 4-, 8- or 12 hours later, sperm were flushed from the excised uteri with fixative. Sperm viability was determined at 1250x magnification under oil using brightfield optics from vitally stained smears prepared from fixed samples of the inseminate and uterine sperm using a staining technique validated as part of the study. Sperm morphology was separately determined from wet mounts examined at 1250x magnification under oil using differential interference contrast optics. Two hundred sperm were counted from each sample to determine both morphology and viability. When data were pooled across times post-insemination, significantly greater levels of live sperm and normal sperm were present in the uterine population as compared to the inseminate. The enhanced level of normal sperm in the uterine population may have been due to cervical or uterine filtration, selective removal in retrograde mucus, preferential phagocytosis or differential death of abnormal vs normal sperm.

In light of the results of Study 1, two subsequent experiments were conducted in Study 2. Using pairs of heifers simultaneously in estrus, 0.5 ml semen containing high levels of

morphologically abnormal sperm was deposited in the uterus of one heifer and 5 ml was vaginally deposited in the other heifer of each pair. Discharged retrograde mucus was collected for 6 hours immediately following insemination and, 12 hours after insemination, heifer pairs were slaughtered and sperm were recovered from the vaginal mucus, the cervix and the uterus of each excised reproductive tract and preserved in glutaraldehyde-paraformaldehyde fixative. A sample of the inseminate was incubated in egg yolk-sodium citrate extender *in vitro* from the time of insemination until sperm were recovered from the uteri as a test for differential sperm death based upon morphology. In Experiment 1, 5 pairs of heifers were inseminated with semen of high viability and in Experiment 2, low viability semen was deposited in 3 pairs of heifers. Sperm head morphology was stringently evaluated in Experiment 1 and more leniently evaluated in Experiment 2 to determine the ability of the cervix to differentiate gross from subtle head abnormalities in the event cervical filtration of sperm based upon morphology became evident. Sperm viability (based upon acrosomal integrity) and sperm morphology were coincidentally determined for each of 200 sperm counted per wet mount examined at 1250x magnification using differential interference contrast optics.

The results of Experiment 1 and Experiment 2 showed that levels of uterine sperm quality traits did not differ between sites of semen deposition. This indicated that uterine sperm quality was not affected by semen deposition site at the time of sampling and offered no evidence of cervical filtration of morphologically abnormal sperm. Reduced uterine levels of live abnormal sperm after intrauterine semen deposition in Experiment 2 suggested a beneficial effect of intrauterine vs vaginal deposition and made trivial the concept of cervical selection of morphologically abnormal sperm. However, this result could have occurred due to exclusion of decapitated sperm, levels of which were relatively high in the inseminate in Experiment 2 vs Experiment 1 and of which most were dead, causing the significant difference between inseminate and uterus. Compared to the inseminate, viability of the uterine sperm population was enhanced and morphology was unchanged after insemination of low viability semen, demonstrating that sperm viability was a major determinant of uterine sperm quality. When high viability semen was used, a subtle effect of sperm morphology on the uterine

sperm quality became apparent with a subtle reduction in uterine viability, enhanced levels of normal sperm, unchanged live normal sperm levels and reduced levels of live abnormal sperm, as compared to the inseminate. These results indicated that sperm viability exerted an overriding effect on sperm morphology in determining the quality of the uterine sperm population. The effects of sperm morphology on uterine sperm quality became evident, albeit subtly, when sperm viability effects were minimized, i.e., when very high viability semen was used.

At the time of sampling, evidence in the cervix indicated the lack of effect that organ had on sperm passing across it. In both experiments, the site of insemination had no effect on levels of sperm traits within it. This was not interpreted as indicating the cervix had no effect on the quality of the sperm passing across it, due to the well established fact that the cervix constitutes a formidable barrier to the passage of nonviable vaginal sperm during sustained transport. Results indicate that sperm were distributed in a predominantly uniform manner along the length of the cervix in the central and peripheral aspects, regardless of morphology and viability. When data were pooled across insemination sites and cervical quarters, a viability gradient was evident between the central and peripheral aspects of the cervix. Greater levels of live sperm and live normal sperm were located between mucosal folds in the peripheral cervix with uniform distribution of other sperm throughout the organ, indicating a preferential colonization of the deeper folds of the cervical mucosa by live normal sperm. The higher viability of the sperm population between the cervical folds may constitute evidence of selection that had occurred prior to sampling.

The limited data on vaginal mucus demonstrated that insemination site had no effect on levels of sperm traits in the vaginal mucus, suggesting that evidence of cervical filtration of sperm, if it had occurred, had disappeared or was not discernible using the methods employed in these studies. Sperm presence in the vaginal mucus appeared to be based upon sperm viability, with reduced levels of viable sperm evident in the vaginal mucus compared to the inseminate and uterine populations. Sperm morphology did not appear to have an effect on sperm presence in the vaginal mucus.

Levels of sperm traits in the retrograde mucus were affected by semen deposition site, and differences appeared to be based upon sperm viability. Regardless of the viability of the semen deposited, after vaginal insemination the sperm population in the retrograde mucus was highly viable, while after intrauterine insemination, the the sperm population it contained was of low viability. The results indicate that retrograde mucus was the vehicle for removal of viable sperm after vaginal insemination and transported nonviable sperm out of the tract after intrauterine insemination. Sperm morphology did not appear to be a basis for sperm presence in retrograde mucus. However, when relatively high levels of decapitated sperm were inseminated, relatively high levels of the abnormality resulted in the retrograde mucus, indicating the importance of sperm motility to their persistance in the female tract.

The results of *in vitro* incubation of inseminate samples demonstrated that the changes in viability of normal and abnormal sperm did not differ. These results showed that differential death of sperm *in vitro* and suggest that it did not occur *in vivo*

Conclusions

Based upon the evidence presented in this study, and in response to the experimental objectives it is concluded:

1) Evidence of a cervical effect on the quality of sperm passing across it towards the uterus had either disappeared by the time of sampling or was not discernible using the methods employed in these studies.

2) Greater levels of live and live normal sperm were located in the peripheral, compared to the more central aspects of the cervical canal with uniform distribution of other forms along the cervix.

3) Sperm quality in the uterus, cervix and vaginal mucus 12 hours after insemination was, with one exception, not affected by site of semen deposition.

4) Intrauterine semen deposition may be beneficial to the quality of the uterine sperm population compared to vaginal insemination, based upon the reduced live abnormal sperm in the uterus after intrauterine semen deposition as compared to vaginal semen deposition. However, disproportionately high levels of one abnormality being dead upon insemination may have affected this result.

5) Based upon the uterine, cervical and vaginal mucus sperm 12 hours after insemination, there appeared to be preferential retention of sperm due to their viability but not morphology.

6) Sperm viability had an overriding influence on sperm presence in the female compared to sperm morphology, whose effects were subtly apparent only when viability effects were minimized.

7) The retrograde mucus contained high levels of viable sperm after vaginal insemination and high levels of nonviable sperm after intrauterine insemination, as compared to the inseminate.

8) Differential death based upon sperm morphology did not occur *in vitro*.

9) Vital staining sperm preserved in glutaraldehyde-paraformaldehyde fixative reproduces the results of vital staining of unfixed (air fixed) sperm when half-stained sperm are counted with unstained sperm as alive.

10) Fixation of sperm prior to vital staining reduced the proportion of half-stained sperm, which appear to be the source of differences between levels of unstained sperm when vital staining results of fixed and unfixed sperm are compared.

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Appendix A. Appendix Tables

Appendix Table 1. Contrasts performed in Preliminary Studies. The Bonferroni t^2 was used to test significance in the event of nonorthogonality.

TRIAL 1.

Fixed vs. unfixed samples before heat (H) kill treatment.
Fixed vs. unfixed samples after heat (H) kill treatment.

Fixed vs. unfixed samples before freeze-thaw (F) kill treatment.
Fixed vs. unfixed samples after freeze-thaw (F) kill treatment.

Fixed vs. unfixed samples before incubation (I) kill treatment.
Fixed vs. unfixed samples after incubation (I) kill treatment.

Among kill methods (H vs. F, H vs. I, F vs. I) before kill treatment for unfixed samples.
Among kill methods (H vs. F, H vs. I, F vs. I) after kill treatment for unfixed samples.

Among kill methods (H vs. F, H vs. I, F vs. I) before kill treatment for fixed samples.
Among kill methods (H vs. F, H vs. I, F vs. I) after kill treatment for fixed samples.

TRIAL 2.

Fixed vs. unfixed samples for each level of killed sperm added.

SPERM TRANSPORT TRIAL.

Inseminate vs. uterine flush at 4 hours post-insemination.
Inseminate vs. uterine flush at 8 hours post-insemination.
Inseminate vs. uterine flush at 12 hours post-insemination.
Inseminate vs. uterine flush pooled across times post-insemination.

Appendix Table 2. Contrasts performed in Study #2 for Experiment 1 and Experiment 2. The Bonferroni t^2 was used to test significance in the event of nonorthogonality.

Inseminate vs. uterine flush for vaginally-inseminated heifers.
Inseminate vs. uterine flush for intrauterine-inseminated heifers.
Inseminate vs uterine flush pooled across insemination sites.

Uterine flushes from vaginally- vs. intrauterine-inseminated heifers.

Among cervical quarters (Qtr.1 vs. Qtr.2, Qtr.1 vs. Qtr.3, Qtr.1 vs Qtr.4, Qtr.2 vs. Qtr.3, Qtr.2 vs. Qtr.4, Qtr.3 vs. Qtr.4) from central cervical canal samples in vaginally-inseminated heifers.
Among cervical quarters (Qtr.1 vs. Qtr.2, Qtr.1 vs. Qtr.3, Qtr.1 vs Qtr.4, Qtr.2 vs. Qtr.3, Qtr.2 vs. Qtr.4, Qtr.3 vs. Qtr.4) from peripheral cervical canal samples in vaginally-inseminated heifers.
Among cervical quarters (Qtr.1 vs. Qtr.2, Qtr.1 vs. Qtr.3, Qtr.1 vs Qtr.4, Qtr.2 vs. Qtr.3, Qtr.2 vs. Qtr.4, Qtr.3 vs. Qtr.4) from central cervical canal samples in intrauterine-inseminated heifers.
Among cervical quarters (Qtr.1 vs. Qtr.2, Qtr.1 vs. Qtr.3, Qtr.1 vs Qtr.4, Qtr.2 vs. Qtr.3, Qtr.2 vs. Qtr.4, Qtr.3 vs. Qtr.4) from peripheral cervical canal samples in intrauterine-inseminated heifers.

Central vs. peripheral cervical canal samples in Qtr.1 for vaginally-inseminated heifers.
Central vs. peripheral cervical canal samples in Qtr.1 for intrauterine-inseminated heifers.
Central vs. peripheral cervical canal samples in Qtr.2 for vaginally-inseminated heifers.
Central vs. peripheral cervical canal samples in Qtr.2 for intrauterine-inseminated heifers.
Central vs. peripheral cervical canal samples in Qtr.3 for vaginally-inseminated heifers.
Central vs. peripheral cervical canal samples in Qtr.3 for intrauterine-inseminated heifers.
Central vs. peripheral cervical canal samples in Qtr.4 for vaginally-inseminated heifers.
Central vs. peripheral cervical canal samples in Qtr.4 for intrauterine-inseminated heifers.

Appendix Table 2. Continued. Contrasts performed in Study #2.

Among cervical quarters (Qtr.1 vs. Qtr.2, Qtr.1 vs. Qtr.3, Qtr.1 vs Qtr.4, Qtr.2 vs. Qtr.3, Qtr.2 vs. Qtr.4, Qtr.3 vs. Qtr.4) from central cervical canal samples pooled across insemination sites.

Among cervical quarters (Qtr.1 vs. Qtr.2, Qtr.1 vs. Qtr.3, Qtr.1 vs Qtr.4, Qtr.2 vs. Qtr.3, Qtr.2 vs. Qtr.4, Qtr.3 vs. Qtr.4) from peripheral cervical canal samples pooled across insemination sites.

Central vs. peripheral cervical canal samples in Qtr.1 pooled across insemination sites.

Central vs. peripheral cervical canal samples in Qtr.2 pooled across inseminated sites.

Central vs. peripheral cervical canal samples in Qtr.3 pooled across insemination sites.

Central vs. peripheral cervical canal samples in Qtr.4 pooled across insemination sites.

Central vs. peripheral cervical canal samples pooled across insemination sites and cervical quarters.

Vaginal mucus vs. inseminate, central cervix, peripheral cervix and uterine flush for vaginally-inseminated heifers.Not performed in Experiment 2 due to missing data.

Vaginal mucus vs. inseminate, central cervix, peripheral cervix and uterine flush for intrauterine-inseminated heifers.

Vaginal mucus vs. inseminate, central cervix, peripheral cervix and uterine flush pooled across insemination sites.

Retrograde mucus vs. inseminate, central cervix, peripheral cervix and uterine flush for vaginally-inseminated heifers.

Retrograde mucus vs. inseminate, central cervix, peripheral cervix and uterine flush for intrauterine-inseminated heifers.

Retrograde mucus vs. inseminate, central cervix, peripheral cervix and uterine flush pooled across insemination sites.

Appendix Table 3. Least squares means ± standard errors for percentages of sperm head abnormalities in the inseminate and uterine flush sperm populations by semen deposition site.[a] Experiment 1.

Sperm Trait	Site	Inseminate	Uterine Flush
Tapered	v	23.4 ± 2.3	22.1 ± 2.3
	u	23.4 ± 2.3	17.2 ± 2.7
Cratered	v	9.3 ± 0.8	8.2 ± 0.8
	u	9.3 ± 0.8	7.3 ± 1.0
Asymmetric	v	5.6 ± 1.2	4.5 ± 1.2
	u	5.6 ± 1.2	5.2 ± 1.3
Decapitated	v	1.6 ± 1.0	2.3 ± 1.0
	u	1.6 ± 1.0	0.6 ± 1.2
Pyriform	v	0.7 ± 0.3	0.2 ± 0.3
	u	0.7 ± 0.3	0.2 ± 0.3
Short	v	0.5 ± 0.2	0.2 ± 0.2
	u	0.5 ± 0.2	0.2 ± 0.2
Flat	v	0.2 ± 0.2	0.4 ± 0.2
	u	0.2 ± 0.2	0.8 ± 0.2
Elongated	v	0.0 ± 0.1	0.1 ± 0.1
	u	0.0 ± 0.1	0.0 ± 0.1

[a]site v = vaginal semen deposition
u = intrauterine semen deposition

Appendix Table 4. Least squares means ± standard errors for percentages of sperm both alive and having head abnormalities in the inseminate and uterine flush[a] sperm populations by semen deposition site.[b] Experiment 1.

Sperm Trait	Site	Inseminate	Uterine Flush
Live Tapered	v	21.3 ± 2.3	18.7 ± 2.3
	u	21.3 ± 2.3	14.0 ± 2.7
Live Cratered	v	8.6 ± 1.4	6.9 ± 1.4
	u	8.6 ± 1.4	6.3 ± 1.6
Live Asymmetric	v	5.4 ± 1.0	3.3 ± 1.0
	u	5.4 ± 1.0	4.7 ± 1.2
Live Decapitated	v	0.4 ± 0.3	1.6 ± 0.3
	u	0.4 ± 0.3	0.1 ± 0.4
Live Pyriform	v	0.5 ± 0.2	0.2 ± 0.2
	u	0.5 ± 0.2	0.0 ± 0.3
Live Short	v	0.5 ± 0.2	0.2 ± 0.2
	u	0.5 ± 0.2	0.0 ± 0.2
Live Flat	v	0.2 ± 0.2	0.4 ± 0.2
	u	0.2 ± 0.2	0.8 ± 0.2
Live Elongated	v	0.0 ± 0.1	0.0 ± 0.1
	u	0.0 ± 0.1	0.0 ± 0.1

[a]Note: this was based upon sperm viability 12 hours after insemination
[b]site v = vaginal semen deposition
u = intrauterine semen deposition

Appendix Table 5. Least squares means ± standard errors for percentages of sperm head abnormalities in the inseminate and uterine flush[a] sperm populations pooled across semen deposition sites. Experiment 1.

Sperm Trait	Inseminate	Uterine Flush
Tapered	23.4 ± 1.6	19.7 ± 1.8
Cratered	9.3 ± 0.6	7.8 ± 0.6
Asymmetric	6.0 ± 0.8	4.9 ± 0.9
Decapitated	1.6 ± 0.7	1.4 ± 0.8
Pyriform	0.7 ± 0.2	0.2 ± 0.2
Short	0.5 ± 0.1	0.1 ± 0.1
Flat	0.2 ± 0.1	0.6 ± 0.2
Elongated	0.0 ± 0.0	0.0 ± 0.0
Live Tapered	21.3 ± 1.7	16.4 ± 1.8
Live Cratered	8.6 ± 1.0	6.6 ± 1.0
Live Asymmetric	5.4 ± 0.7	4.0 ± 0.8
Live Decapitated	0.4 ± 0.2	0.9 ± 0.3
Live Pyriform	0.5 ± 0.2	0.1 ± 0.2
Live Short	0.5 ± 0.1	0.1 ± 0.1
Live Flat	0.2 ± 0.1	0.6 ± 0.2
Live Elongated	0.0 ± 0.0	0.0 ± 0.0

[a]Note: this was based upon sperm quality 12 hours after insemination

Appendix Table 6. Least squares means ± standard errors for percentages of sperm quality traits in samples taken from cervical quarters by insemination site[a], cervical region[b], and cervical quarter[c]. Experiment 1.

Sperm Trait	Site	Cervical Region							
		Central				Peripheral			
		Qtr1	Qtr2	Qtr3	Qtr4	Qtr1	Qtr2	Qtr3	Qtr4
Line	v	64.4 ± 5.2	67.7 ± 4.5	68.6 ± 4.5	80.6 ± 4.5	75.9 ± 4.5	80.3 ± 4.5	78.7 ± 5.2	84.1 ± 4.5
	u	49.2 ± 7.9	45.4 ± 7.9	67.7 ± 4.5	67.1 ± 6.5	71.2 ± 5.6	62.8 ± 6.5	80.4 ± 5.6	80.4 ± 6.5
Normal	v	65.1 ± 2.7	59.1 ± 2.4	61.7 ± 2.4	64.8 ± 2.4	65.5 ± 2.4	67.8 ± 2.4	67.0 ± 2.7	63.9 ± 2.4
	u	62.8 ± 4.1	62.5 ± 4.1	64.8 ± 2.4	57.8 ± 3.4	66.4 ± 2.9	61.6 ± 3.4	62.9 ± 2.9	61.6 ± 3.4
Abnormal	v	35.0 ± 2.7	41.3 ± 2.4	38.7 ± 2.4	35.5 ± 2.4	34.7 ± 2.4	32.4 ± 2.4	33.1 ± 2.7	36.1 ± 2.4
	u	37.3 ± 4.1	37.6 ± 4.1	35.3 ± 2.4	42.3 ± 3.4	33.6 ± 2.9	38.5 ± 3.4	37.1 ± 2.9	38.5 ± 3.4
Primary[d]	v	34.9 ± 2.7	40.9 ± 2.4	38.3 ± 2.4	35.2 ± 2.4	34.5 ± 2.4	32.2 ± 2.4	33.0 ± 2.7	36.1 ± 2.4
	u	37.2 ± 4.1	37.5 ± 4.1	35.2 ± 2.4	42.2 ± 3.4	33.6 ± 2.9	38.4 ± 3.4	37.1 ± 2.9	38.4 ± 3.4
Live Normal	v	41.0 ± 3.2	42.0 ± 2.8	42.0 ± 2.8	54.8 ± 2.8	50.7 ± 2.8	55.9 ± 2.8	53.6 ± 3.2	53.9 ± 2.8
	u	33.3 ± 4.9	32.9 ± 4.9	44.8 ± 2.8	39.2 ± 4.1	49.2 ± 3.4	41.1 ± 4.1	51.4 ± 3.5	51.9 ± 4.1
Live Abnormal	v	23.5 ± 3.5	26.1 ± 3.0	26.9 ± 3.0	26.0 ± 3.0	25.4 ± 3.0	24.7 ± 3.0	25.3 ± 3.5	30.2 ± 3.0
	u	15.9 ± 5.3	12.4 ± 5.3	23.0 ± 3.3	27.9 ± 4.4	22.0 ± 3.7	21.7 ± 4.4	29.0 ± 3.7	28.5 ± 4.4
Live Primary[e]	v	23.4 ± 3.4	25.8 ± 3.0	26.6 ± 3.0	25.9 ± 3.0	25.2 ± 3.0	24.5 ± 3.0	25.2 ± 3.4	30.2 ± 3.0
	u	15.9 ± 5.3	12.4 ± 5.3	23.0 ± 3.0	28.0 ± 4.3	22.0 ± 3.7	21.7 ± 4.3	29.1 ± 3.7	28.5 ± 4.3

[a]Insemination site v = vaginal semen deposition
u = intrauterine semen deposition

[b]Cervical region: Central = obtained by curettage of cervical mucosa proximal to cervical canal
Peripheral = obtained by vacuum deeper between 1° and 2° folds of cervical mucosa

[c]Cervical quarter: Qtr. numbered sequentially 1-4 from vaginal to uterine aspect

[d]Percentage of sperm having abnormal head morphology

[e]Percentage of sperm both alive and having abnormal head morphology

Appendix Table 7. Least squares means ± standard errors for percentages of sperm quality traits in samples taken from cervical quarters by insemination site[a], cervical region[b], and cervical quarter[c]. Experiment 1.

Sperm Trait	Site	Cervical Region							
		Central				Peripheral			
		Qtr1	Qtr2	Qtr3	Qtr4	Qtr1	Qtr2	Qtr3	Qtr4
Tapered	v	21.7 ± 1.9	24.1 ± 1.7	24.8 ± 1.7	20.6 ± 1.7	18.5 ± 1.7	17.5 ± 1.7	17.4 ± 1.9	22.8 ± 1.7
	u	19.7 ± 3.0	21.5 ± 3.0	22.4 ± 1.7	27.5 ± 2.4	21.9 ± 2.1	19.8 ± 2.4	20.3 ± 2.1	22.4 ± 2.4
Cratered	v	6.6 ± 1.4	7.4 ± 1.2	6.4 ± 1.2	7.1 ± 1.2	8.6 ± 1.2	9.0 ± 1.2	7.4 ± 1.4	5.1 ± 1.2
	u	11.1 ± 2.2	9.2 ± 2.2	4.5 ± 1.2	5.4 ± 1.8	7.1 ± 1.5	9.8 ± 1.8	8.4 ± 1.5	5.8 ± 1.8
Asymmetric	v	3.3 ± 1.2	4.9 ± 1.1	4.6 ± 1.1	2.6 ± 1.1	5.0 ± 1.1	4.6 ± 1.1	4.1 ± 1.2	4.1 ± 1.1
	u	3.1 ± 1.9	4.6 ± 1.9	5.1 ± 1.1	6.3 ± 1.5	2.7 ± 1.3	4.6 ± 1.5	6.8 ± 1.3	5.5 ± 1.5
Decapitated	v	1.3 ± 1.1	3.9 ± 1.0	1.8 ± 1.0	3.7 ± 1.0	1.7 ± 1.0	0.8 ± 1.0	2.9 ± 1.1	2.6 ± 1.0
	u	1.4 ± 1.7	1.9 ± 1.7	1.8 ± 1.0	2.3 ± 1.4	1.3 ± 1.2	2.4 ± 1.4	1.1 ± 1.2	4.3 ± 1.4
Pyriform	v	0.7 ± 0.2	0.3 ± 0.2	0.1 ± 0.2	0.3 ± 0.2	0.0 ± 0.2	0.0 ± 0.2	0.7 ± 0.2	0.2 ± 0.2
	u	0.8 ± 0.3	0.3 ± 0.3	0.1 ± 0.2	0.1 ± 0.3	0.1 ± 0.2	0.4 ± 0.3	0.3 ± 0.2	0.1 ± 0.3
Short	v	0.4 ± 0.2	0.2 ± 0.2	0.0 ± 0.2	0.2 ± 0.2	0.0 ± 0.2	0.1 ± 0.2	0.0 ± 0.2	0.6 ± 0.2
	u	0.5 ± 0.3	0.0 ± 0.3	0.3 ± 0.2	0.3 ± 0.3	0.3 ± 0.2	0.0 ± 0.3	0.1 ± 0.2	0.2 ± 0.3
Flat	v	0.2 ± 0.2	0.1 ± 0.1	0.0 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.1
	u	0.1 ± 0.2	0.2 ± 0.2	0.3 ± 0.1	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.0 ± 0.2
Elongated	v	0.2 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.1	0.4 ± 0.4
	u	0.6 ± 0.2	0.1 ± 0.2	0.0 ± 0.1	0.1 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.2 ± 0.2	0.3 ± 0.2

[a]Insemination site v = vaginal semen deposition
u = intrauterine semen deposition

[b]Sample location: Central = obtained by curettage of cervical mucosa proximal to cervical canal
Peripheral = obtained by vacuum deeper between 1° and 2° folds of cervical mucosa

[c]Cervical quarter: Qtr. numbered sequentially 1-4 from vaginal to uterine aspect

Appendix Table 8. Least squares means ± standard errors for percentages of sperm both alive and having head abnormalities in samples taken from cervical quarters by insemination site[a], cervical region[b], and cervical quarter[c]. Experiment 1.

		Cervical Region							
Sperm Trait	Site	Central				Peripheral			
		Qtr1	Qtr2	Qtr3	Qtr4	Qtr1	Qtr2	Qtr3	Qtr4
Live Tapered	v	15.3 ± 2.1	15.8 ± 1.8	16.9 ± 1.8	15.0 ± 1.8	14.1 ± 1.8	12.9 ± 1.8	13.7 ± 2.1	19.8 ± 1.8
	u	10.5 ± 3.2	9.0 ± 3.2	14.8 ± 1.8	18.9 ± 2.6	15.1 ± 2.2	12.6 ± 2.6	15.9 ± 2.2	15.8 ± 2.6
Live Cratered	v	3.4 ± 1.1	3.9 ± 1.0	4.5 ± 1.0	5.6 ± 1.0	6.3 ± 1.0	6.9 ± 1.0	5.8 ± 1.1	4.3 ± 1.0
	u	3.9 ± 1.7	3.4 ± 1.7	3.2 ± 1.0	2.9 ± 1.4	4.8 ± 1.2	5.4 ± 1.4	7.0 ± 1.2	4.9 ± 1.4
Live Asymmetric	v	2.9 ± 1.1	3.5 ± 1.0	3.2 ± 1.0	2.1 ± 1.0	3.3 ± 1.0	4.0 ± 1.0	3.0 ± 1.1	3.6 ± 1.0
	u	0.8 ± 1.7	0.5 ± 1.7	3.8 ± 1.0	5.0 ± 1.4	1.7 ± 1.2	2.6 ± 1.4	5.5 ± 1.2	5.0 ± 1.4
Live Decapitated	v	0.9 ± 0.8	2.2 ± 0.7	1.3 ± 0.7	2.2 ± 0.7	0.9 ± 0.7	0.5 ± 0.7	2.2 ± 0.8	1.5 ± 0.7
	u	0.2 ± 1.2	0.0 ± 1.2	0.3 ± 0.7	0.5 ± 1.0	0.1 ± 0.9	0.1 ± 1.0	0.3 ± 0.9	2.3 ± 1.1
Live Pyriform	v	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
	u	0.1 ± 0.2	0.4 ± 0.2	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.2	0.3 ± 0.2	0.1 ± 0.2	0.1 ± 0.2
Live Short	v	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.2 ± 0.1
	u	0.1 ± 0.2	0.0 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.2	0.0 ± 0.2	0.1 ± 0.2	0.2 ± 0.2
Live Flat	v	0.2 ± 0.2	0.1 ± 0.1	0.0 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.1
	u	0.1 ± 0.2	0.2 ± 0.2	0.3 ± 0.1	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.2	0.0 ± 0.2
Live Elongated	v	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.1	0.3 ± 0.1
	u	0.6 ± 0.2	0.1 ± 0.2	0.0 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.1

[a]Insemination site v = vaginal semen deposition u = intrauterine semen deposition

[b]Cervical region: Central = obtained by curettage of cervical mucosa proximal to cervical canal
Peripheral = obtained by vacuum deeper between 1° and 2° folds of cervical mucosa

[c]Cervical quarter: Qtr. numbered sequentially 1-4 from vaginal to uterine aspect

Appendix Table 9. Least squares means ± standard errors for percentages of sperm quality traits in samples taken from cervical quarters by cervical region[a] and cervical quarter[b] pooled across site of semen deposition. Experiment 1.

	Cervical Region							
Sperm Trait	Central				Peripheral			
	Qtr1	Qtr2	Qtr3	Qtr4	Qtr1	Qtr2	Qtr3	Qtr4
Live	56.8 ± 4.7	56.5 ± 4.6	68.2 ± 3.2	73.9 ± 4.0	73.6 ± 3.6	71.6 ± 4.0	79.6 ± 3.8	82.2 ± 4.0
Normal	64.0 ± 2.5	60.8 ± 2.4	63.2 ± 1.7	61.3 ± 2.1	65.9 ± 1.9	64.4 ± 2.1	64.9 ± 2.0	62.8 ± 2.1
Abnormal	36.1 ± 2.5	39.5 ± 2.4	37.0 ± 1.7	38.9 ± 2.1	34.2 ± 1.9	35.4 ± 2.1	35.1 ± 2.0	37.3 ± 2.1
Primary[c]	36.0 ± 2.5	39.2 ± 2.4	36.8 ± 1.7	38.7 ± 2.1	34.1 ± 1.9	35.3 ± 2.1	35.1 ± 2.0	37.2 ± 2.1
Live Normal	37.2 ± 3.0	37.5 ± 2.9	43.3 ± 2.0	47.0 ± 2.5	49.9 ± 2.2	48.5 ± 2.5	52.5 ± 2.4	52.9 ± 2.5
Live Abnormal	19.7 ± 3.2	19.3 ± 3.0	25.0 ± 2.1	26.9 ± 2.7	23.7 ± 2.4	23.2 ± 2.7	27.1 ± 2.5	29.3 ± 2.7
Live Primary[d]	19.6 ± 3.1	19.1 ± 3.0	24.8 ± 2.1	26.8 ± 2.6	23.6 ± 2.4	23.1 ± 2.6	27.1 ± 2.5	29.3 ± 2.6

[a]Cervical region: Central: obtained by curettage of cervical mucosa proximal to cervical canal
Peripheral: obtained by vacuum deeper between 1° and 2° folds of cervical mucosa

[b]Cervical quarter: Qtr. numbered sequentially 1-4 from vaginal to uterine aspect

[c]Percentage of sperm having abnormal head morphology

[d]Percentage of sperm both alive and having abnormal head morphology

Appendix Table 10. Least squares means ± standard errors for percentages of sperm head abnormalities in samples taken from cervical quarters by cervical region[a] and cervical quarter[b] pooled across site of semen deposition. Experiment 1.

	Cervical Region							
Sperm Trait	Central				Peripheral			
	Qtr1	Qtr2	Qtr3	Qtr4	Qtr1	Qtr2	Qtr3	Qtr4
Tapered	20.7 ± 1.8	22.8 ± 1.7	23.6 ± 1.2	24.0 ± 1.5	20.2 ± 1.3	18.6 ± 1.5	18.9 ± 1.4	22.6 ± 1.5
Cratered	8.8 ± 1.3	8.3 ± 1.3	5.4 ± 1.0	6.2 ± 1.1	7.9 ± 1.0	9.4 ± 1.1	7.9 ± 1.0	5.4 ± 1.1
Asymmetric	3.2 ± 1.1	4.7 ± 1.1	4.9 ± 0.8	4.4 ± 0.9	3.9 ± 0.8	4.6 ± 0.9	5.4 ± 0.9	4.8 ± 0.9
Decapitated	1.3 ± 1.0	2.9 ± 1.0	1.8 ± 0.7	3.0 ± 0.9	1.5 ± 0.8	1.6 ± 0.9	2.0 ± 0.8	3.4 ± 0.9
Pyriform	0.8 ± 0.2	0.3 ± 0.2	0.1 ± 0.1	0.2 ± 0.2	0.5 ± 0.2	0.2 ± 0.2	0.5 ± 0.2	0.1 ± 0.2
Short	0.4 ± 0.2	0.1 ± 0.2	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.2	0.0 ± 0.2	0.4 ± 0.2
Flat	0.1 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.1
Elongated	0.4 ± 0.1	0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.1
Live Tapered	12.9 ± 1.9	12.4 ± 1.8	15.9 ± 1.3	16.9 ± 1.6	14.6 ± 1.4	12.7 ± 1.6	14.8 ± 1.5	17.8 ± 1.6
Live Cratered	3.7 ± 1.0	3.7 ± 1.0	3.8 ± 0.7	4.3 ± 0.9	5.6 ± 0.8	6.1 ± 0.9	6.4 ± 0.8	4.6 ± 0.9
Live Asymmetric	1.8 ± 1.0	1.5 ± 1.0	3.5 ± 0.7	3.5 ± 0.8	2.5 ± 0.8	3.3 ± 0.8	4.2 ± 0.8	4.3 ± 0.8
Live Decapitated	0.3 ± 0.7	1.1 ± 0.7	0.8 ± 0.5	1.3 ± 0.6	0.4 ± 0.6	0.3 ± 0.6	1.3 ± 0.6	1.9 ± 0.6
Live Pyriform	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Live Short	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.2 ± 0.1
Live Flat	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.1
Live Elongated	0.3 ± 0.1	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.6 ± 0.1	0.3 ± 0.1

[a]Cervical region: Central: obtained by curettage of cervical mucosa proximal to cervical canal
Peripheral: obtained by vacuum deeper between 1° and 2° folds of cervical mucosa

[b]Cervical quarter: Qtr. numbered sequentially 1-4 from vaginal to uterine aspect

Appendix Table 11. Least squares means ± standard errors for percentages of sperm head abnormalities in the cervical sperm population by cervical region[a] and pooled across cervical quarters and semen deposition site. Experiment 1.

Sperm Trait	Cervical Region	
	Central	Peripheral
Tapered	21.3 ± 0.4	18.4 ± 0.3**
Cratered	9.9 ± 0.4	10.1 ± 0.3
Asymmetric	5.6 ± 0.5	5.4 ± 0.4
Decapitated	1.6 ± 0.7	1.6 ± 0.5
Pyriform	0.4 ± 0.2	0.3 ± 0.1
Short	0.6 ± 0.2	0.1 ± 0.1
Flat	0.1 ± 0.1	0.2 ± 0.1
Elongated	0.1 ± 0.1	0.3 ± 0.1
Live Tapered	13.6 ± 0.9	13.9 ± 0.7
Live Cratered	5.8 ± 0.7	7.4 ± 0.6
Live Asymmetric	3.8 ± 0.4	4.3 ± 0.3
Live Decapitated	0.8 ± 0.5	1.0 ± 0.4
Live Pyriform	0.2 ± 0.1	0.2 ± 0.1
Live Short	0.6 ± 0.1	0.1 ± 0.1*
Live Flat	0.1 ± 0.1	0.1 ± 0.1
Live Elongated	0.0 ± 0.1	0.2 ± 0.1

[a]Sample location: Central: obtained by curettage of cervical mucosa proximal to cervical canal
Peripheral: obtained by vacuum deeper between 1° and 2° folds of cervical mucosa

*Means within lines differ ($p<.05$)
**Means within lines differ ($p<.01$)

Appendix Table 12. Least squares means ± standard errors for percentages of sperm head abnormalities in the vaginal mucus sperm population compared to those in other sample locations by insemination site.[a] Experiment 1.

Sperm Trait	Site	Vaginal Mucus	Inseminate	Cervix		Uterus
				Central	Peripheral	
Tapered	v	24.9 ± 2.7	23.4 ± 2.0	24.3 ± 2.3	20.6 ± 2.3	22.1 ± 2.0
	u	21.9 ± 2.7	23.4 ± 2.0	22.5 ± 3.4	20.4 ± 2.8	17.2 ± 2.3
Cratered	v	8.5 ± 1.1	9.3 ± 0.8	6.8 ± 1.0	7.6 ± 1.0	8.2 ± 0.8
	u	6.9 ± 1.3	9.3 ± 0.8	7.7 ± 1.4	6.1 ± 1.1	7.3 ± 1.0
Asymmetric	v	4.4 ± 1.4	6.0 ± 1.1	4.0 ± 1.2	4.6 ± 1.2	4.5 ± 1.1
	u	7.8 ± 1.4	6.0 ± 1.1	5.9 ± 1.8	5.4 ± 1.4	5.2 ± 1.2
Decapitated	v	4.5 ± 1.2	1.6 ± 0.9	3.3 ± 1.0	2.4 ± 1.0	2.3 ± 0.9
	u	2.2 ± 1.2	1.6 ± 0.9	1.3 ± 1.5	1.7 ± 1.2	0.6 ± 1.1
Pyriform	v	0.3 ± 0.3	0.7 ± 0.2	0.3 ± 0.3	0.2 ± 0.3	0.2 ± 0.2
	u	0.4 ± 0.3	0.7 ± 0.2	0.1 ± 0.4	0.1 ± 0.4	0.2 ± 0.3
Short	v	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.1 ± 0.2	0.2 ± 0.2
	u	0.2 ± 0.2	0.5 ± 0.2	0.5 ± 0.3	0.0 ± 0.2	0.2 ± 0.2
Flat	v	0.3 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.2	0.4 ± 0.2
	u	0.0 ± 0.2	0.2 ± 0.2	0.4 ± 0.3	0.4 ± 0.2	0.8 ± 0.2
Elongated	v	0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
	u	0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	0.0 ± 0.1

[a]Insemination site v = vaginal semen deposition
u = intrauterine semen deposition

Appendix Table 13. Least squares means ± standard errors for percentages of sperm both alive and having head abnormalities in the vaginal mucus sperm population compared to those in other sample locations by insemination site.[a] Experiment 1.

Sperm Trait	Site	Vaginal Mucus	Inseminate	Cervix		Uterus
				Central	Peripheral	
Live Tapered	v	12.0 ± 2.8	21.3 ± 2.1	15.9 ± 2.4	15.7 ± 2.4	18.7 ± 2.1
	u	11.0 ± 2.8	21.3 ± 2.1	12.6 ± 3.5	14.3 ± 2.8	14.0 ± 2.4
Live Cratered	v	6.1 ± 1.7	8.6 ± 1.2	4.0 ± 1.4	5.7 ± 1.4	6.9 ± 1.2
	u	5.4 ± 1.7	8.6 ± 1.2	2.7 ± 2.1	3.8 ± 1.7	6.3 ± 1.4
Live Asymmetric	v	2.7 ± 1.2	5.4 ± 0.9	3.0 ± 1.1	3.6 ± 1.1	3.3 ± 0.9
	u	3.2 ± 1.3	5.4 ± 0.9	3.4 ± 1.6	4.5 ± 1.3	4.7 ± 1.1
Live Decapitate	v	1.1 ± 0.5	0.4 ± 0.4	1.9 ± 0.4	1.4 ± 0.4	1.6 ± 0.4
	u	0.1 ± 0.5	0.4 ± 0.4	0.1 ± 0.6	0.7 ± 0.5	0.1 ± 0.4
Live Pyriform	v	0.2 ± 0.3	0.5 ± 0.2	0.1 ± 0.2	0.0 ± 0.2	0.2 ± 0.2
	u	0.4 ± 0.3	0.5 ± 0.2	0.2 ± 0.4	0.1 ± 0.3	0.0 ± 0.2
Live Short	v	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.0 ± 0.2	0.2 ± 0.2
	u	0.1 ± 0.2	0.5 ± 0.2	0.5 ± 0.3	0.0 ± 0.2	0.0 ± 0.2
Live Flat	v	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.2	0.4 ± 0.2
	u	0.2 ± 0.2	0.2 ± 0.2	0.4 ± 0.3	0.4 ± 0.2	0.8 ± 0.2
Live Elongated	v	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	0.0 ± 0.1
	u	0.0 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.0 ± 0.1

[a]Insemination site v = vaginal semen deposition
u = intrauterine semen deposition

Appendix Table 14. Least squares means ± standard errors for percentages of sperm both alive and having specific head abnormalities in the retrograde mucus sperm population compared to those in other sample locations, by insemination site.[a] Experiment 1.

Sperm Trait	Site	Retrograde Mucus	Inseminate	Cervix		Uterus
				Central	Peripheral	
Live Tapered	v	22.6 ± 2.1	21.3 ± 2.1	15.9 ± 2.4	15.7 ± 2.4	18.7 ± 2.1
	u	8.1 ± 2.4[++]	21.3 ± 2.1[**]	12.6 ± 3.5	14.3 ± 2.8	14.0 ± 2.4
Live Cratered	v	5.5 ± 1.2	8.6 ± 1.2	4.0 ± 1.4	5.7 ± 1.4	6.9 ± 1.2
	u	3.0 ± 1.4	8.6 ± 1.2	2.7 ± 2.1	3.8 ± 1.7	6.3 ± 1.4
Live Asymmetric	v	4.7 ± 0.9	5.4 ± 0.9	3.0 ± 1.1	3.6 ± 1.1	3.3 ± 0.9
	u	3.7 ± 1.1	5.4 ± 0.9	3.4 ± 1.6	4.5 ± 1.3	4.7 ± 1.1
Live Decapitated	v	0.2 ± 0.4	0.4 ± 0.4	1.9 ± 0.4	1.4 ± 0.4	1.6 ± 0.4
	u	0.2 ± 0.4	0.4 ± 0.4	0.1 ± 0.6	0.7 ± 0.5	0.1 ± 0.4
Live Pyriform	v	0.3 ± 0.2	0.5 ± 0.2	0.1 ± 0.2	0.0 ± 0.2	0.2 ± 0.2
	u	0.8 ± 0.2	0.5 ± 0.2	0.2 ± 0.4	0.1 ± 0.3	0.0 ± 0.2
Live Short	v	0.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.0 ± 0.2	0.2 ± 0.2
	u	0.1 ± 0.2	0.5 ± 0.2	0.5 ± 0.3	0.0 ± 0.2	0.0 ± 0.2
Live Flat	v	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.2	0.4 ± 0.2
	u	0.3 ± 0.2	0.2 ± 0.2	0.4 ± 0.3	0.4 ± 0.2	0.8 ± 0.2
Live Elongated	v	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	0.0 ± 0.1
	u	0.0 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.0 ± 0.1

[a]Insemination site v = vaginal semen deposition
u = intrauterine semen deposition

[**]Differs from retrograde mucus mean ($p<.01$)

[++]Column means within sperm trait differ ($p<.01$)

Appendix Table 15. Least squares means ± standard errors for percentages of sperm having specific head abnormalities in the retrograde mucus sperm population compared to those in other sample locations, by insemination site.[a] Experiment 1.

Sperm Trait	Site	Retrograde Mucus	Inseminate	Cervix		Uterus
				Central	Peripheral	
Tapered	v	27.0 ± 2.0	23.4 ± 2.0	24.3 ± 2.3	20.6 ± 2.3	22.1 ± 2.0
	u	20.3 ± 2.3	23.4 ± 2.0	22.5 ± 3.4	20.4 ± 2.8	17.2 ± 2.3
Cratered	v	6.6 ± 0.8	9.3 ± 0.8	6.8 ± 1.0	7.6 ± 1.0	8.2 ± 0.8
	u	6.7 ± 1.0	9.3 ± 0.8	7.7 ± 1.4	6.1 ± 1.1	7.3 ± 1.0
Asymmetric	v	5.7 ± 1.1	6.0 ± 1.1	4.0 ± 1.2	4.6 ± 1.2	4.5 ± 1.1
	u	6.3 ± 1.2	6.0 ± 1.1	5.9 ± 1.8	5.4 ± 1.4	5.2 ± 1.2
Decapitated	v	0.9 ± 0.9	1.6 ± 0.9	3.3 ± 1.0	2.4 ± 1.0	2.3 ± 0.9
	u	1.7 ± 1.1	1.6 ± 0.9	1.3 ± 1.5	1.7 ± 1.2	0.6 ± 1.1
Pyriform	v	0.4 ± 0.2	0.7 ± 0.2	0.3 ± 0.3	0.2 ± 0.3	0.2 ± 0.3
	u	1.5 ± 0.3	0.7 ± 0.3	0.1 ± 0.4	0.1 ± 0.4	0.2 ± 0.3
Short	v	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.1 ± 0.2	0.2 ± 0.2
	u	0.1 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.0 ± 0.2	0.2 ± 0.2
Flat	v	0.3 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.2	0.4 ± 0.2
	u	0.3 ± 0.2	0.2 ± 0.2	0.4 ± 0.3	0.4 ± 0.2	0.8 ± 0.2
Elongated	v	0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
	u	0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	0.0 ± 0.1

[a]Insemination site v = vaginal semen deposition
u = intrauterine semen deposition

Appendix Table 16. Least squares means ± standard errors for percentages of sperm head abnormalities in the retrograde mucus sperm population compared to those in other sample locations pooled across insemination site. Experiment 1.

Sperm Trait	Retrograde Mucus	Inseminate	Cervix[a]		Uterus
			Central	Peripheral	
Tapered	23.6 ± 1.5	23.4 ± 1.4	23.4 ± 2.1	20.5 ± 1.5	19.7 ± 1.5
Cratered	6.6 ± 0.6	9.2 ± 0.6*	7.2 ± 0.9	6.8 ± 0.7	7.8 ± 0.6
Asymmetric	6.0 ± 0.8	6.0 ± 0.7	5.0 ± 1.1	5.0 ± 0.9	4.9 ± 0.8
Decapitated	1.3 ± 0.7	1.6 ± 0.6	2.3 ± 0.9	2.0 ± 0.8	1.4 ± 0.7
Pyriform	1.0 ± 0.2	0.7 ± 0.2	0.1 ± 0.2*	0.0 ± 0.2*	0.2 ± 0.2
Short	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.1 ± 0.2	0.2 ± 0.1
Flat	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.6 ± 0.1
Elongated	0.1 ± 0.04	0.0 ± 0.04	0.1 ± 0.1	0.2 ± 0.1	0.0 ± 0.04
Live Tapered	15.3 ± 1.6	21.3 ± 1.5	14.3 ± 2.1	15.0 ± 1.8	16.4 ± 1.6
Live Cratered	4.2 ± 0.9	8.6 ± 0.9*	3.3 ± 1.3	4.8 ± 1.1	6.6 ± 1.0
Live Asymmetric	4.2 ± 0.7	5.4 ± 0.7	3.2 ± 0.9	4.1 ± 0.8	4.0 ± 0.7
Live Decapitated	0.2 ± 0.3	0.4 ± 0.3	1.0 ± 0.4	1.1 ± 0.3	0.9 ± 0.3
Live Pyriform	0.5 ± 0.2	0.5 ± 0.2	0.1 ± 0.2	0.0 ± 0.2	0.1 ± 0.2*
Live Short	0.2 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.0 ± 0.1	0.1 ± 0.1
Live Flat	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.2	0.6 ± 0.1
Live Elongated	0.0 ± 0.04	0.0 ± 0.04	0.1 ± 0.1	0.2 ± 0.1	0.0 ± 0.04

[a]Central cervix = obtained by curettage of cervical mucosa proximal to cervical canal
Peripheral cervix = obtained by vacuum deeper between 1° and 2° folds of cervical mucosa
*Differs from retrograde mucus mean ($p<.05$)

Appendix Table 17. Least squares means ± standard errors for percentages of sperm both alive and having specific head abnormalities in the inseminate and uterine flush sperm populations by semen deposition site.[a] Experiment 2.

Sperm Trait	Site	Inseminate	Uterine Flush
Live Tapered	v	2.7 ± 2.3	13.9 ± 2.3
	u	2.7 ± 2.3	7.5 ± 2.3
Live Decapitated	v	0.8 ± 0.5	0.0 ± 0.5
	u	0.8 ± 0.5	0.3 ± 0.5
Live Flat	v	2.0 ± 0.9	4.5 ± 0.9
	u	2.0 ± 0.9	2.2 ± 0.9
Live Elongated	v	1.5 ± 1.0	3.3 ± 1.0
	u	1.5 ± 1.0	2.0 ± 1.0
Live Cratered	v	1.2 ± 0.4	2.0 ± 0.4
	u	1.2 ± 0.4	1.8 ± 0.4
Live Pyriform	v	0.7 ± 0.3	0.7 ± 0.3
	u	0.7 ± 0.3	0.5 ± 0.3
Live Asymmetric	v	0.3 ± 0.8	2.8 ± 0.8
	u	0.3 ± 0.8	0.2 ± 0.8
Live Short	v	0.3 ± 0.2	0.0 ± 0.2
	u	0.3 ± 0.2	0.0 ± 0.2

[a]site v = vaginal semen deposition
u = intrauterine semen deposition

Appendix Table 18. Least squares means ± standard errors for percentages of sperm traits in the inseminate and uterine flush sperm populations pooled across semen deposition sites. Experiment 2.

Sperm Trait	Inseminate	Uterine Flush
Decapitated	12.3 ± 4.0	2.7 ± 4.0
Tapered	7.2 ± 1.5	12.6 ± 1.5
Flat	4.0 ± 0.7	4.1 ± 0.7
Elongated	3.5 ± 0.9	3.7 ± 0.9
Cratered	3.3 ± 0.5	3.2 ± 0.5
Pyriform	1.3 ± 0.2	0.8 ± 0.2
Asymmetric	0.5 ± 0.6	1.5 ± 0.6
Short	0.5 ± 0.2	0.1 ± 0.2
Live Decapitated	0.8 ± 0.4	0.2 ± 0.4
Live Tapered	2.7 ± 1.7	10.7 ± 1.7*
Live Flat	2.0 ± 0.7	3.3 ± 0.7
Live Elongated	1.5 ± 0.7	2.7 ± 0.7
Live Cratered	1.2 ± 0.3	1.9 ± 0.3
Live Pyriform	0.7 ± 0.2	0.6 ± 0.2
Live Asymmetric	0.3 ± 0.6	1.5 ± 0.6
Live Short	0.3 ± 0.1	0.0 ± 0.1

*means within lines differ ($p<.05$).

Appendix Table 19. Least squares means ± standard errors for percentages of sperm quality traits in the cervix by insemination site[a], cervical region[b], and cervical quarter[c]. Experiment 2.

Sperm Trait	Site	Cervical Region							
		Central				Peripheral			
		Qtr1	Qtr2	Qtr3	Qtr4	Qtr1	Qtr2	Qtr3	Qtr4
Live	v	35.1 ± 6.4	42.5 ± 6.4	53.2 ± 6.4	51.5 ± 6.4	62.3 ± 6.4	59.2 ± 6.4	54.7 ± 6.4	75.0 ± 6.4
	u	41.7 ± 6.4	47.6 ± 6.4	43.3 ± 6.4	52.4 ± 6.4	58.3 ± 6.4	59.3 ± 6.4	56.3 ± 6.4	49.4 ± 6.4
Normal	v	70.7 ± 4.4	75.7 ± 4.4	72.5 ± 4.4	74.8 ± 4.4	71.9 ± 4.4	74.7 ± 4.4	72.8 ± 4.4	68.9 ± 4.4
	u	76.3 ± 4.4	65.8 ± 4.4	72.3 ± 4.4	74.1 ± 4.4	68.2 ± 4.4	65.5 ± 4.4	74.3 ± 4.4	73.4 ± 4.4
Abnormal	v	29.3 ± 4.4	24.3 ± 4.4	27.5 ± 4.4	25.2 ± 4.4	28.1 ± 4.4	25.3 ± 4.4	27.2 ± 4.4	31.1 ± 4.4
	u	23.8 ± 4.4	34.2 ± 4.4	27.7 ± 4.4	25.9 ± 4.4	31.8 ± 4.4	34.5 ± 4.4	25.7 ± 4.4	26.6 ± 4.4
Live Normal	v	24.3 ± 4.8	31.8 ± 4.8	36.7 ± 4.8	37.2 ± 4.8	43.4 ± 4.8	44.2 ± 4.8	40.8 ± 4.8	53.8 ± 4.8
	u	29.2 ± 4.8	27.6 ± 4.8	30.8 ± 4.8	37.8 ± 4.8	40.9 ± 4.8	38.3 ± 4.8	40.8 ± 4.8	36.6 ± 4.8
Live Abnormal	v	10.8 ± 3.7	10.7 ± 3.7	16.5 ± 3.7	14.3 ± 3.7	19.0 ± 3.7	15.0 ± 3.7	13.8 ± 3.7	21.2 ± 3.7
	u	12.5 ± 3.7	20.0 ± 3.7	12.5 ± 3.7	14.6 ± 3.7	17.3 ± 3.7	21.0 ± 3.7	15.5 ± 3.7	12.8 ± 3.7

[a]site v = vaginal semen deposition
u = intrauterine semen deposition

[b]Cervical region: Central: obtained by curettage of cervical mucosa proximal to cervical canal
Peripheral: obtained by vacuum deeper between 1° and 2° folds of cervical mucosa

[c]Cervical quarter: Qtr. numbered sequentially 1-4 from vaginal to uterine aspect

Appendix Table 20. Least squares means ± standard errors for percentages of sperm head abnormalities in the cervix by insemination site[a], cervical region[b], and cervical quarter[c]. Experiment 2.

Sperm Trait	Site	Cervical Region							
		Central				Peripheral			
		Qtr1	Qtr2	Qtr3	Qtr4	Qtr1	Qtr2	Qtr3	Qtr4
Tapered	v	12.5 ± 3.4	13.7 ± 3.4	15.0 ± 3.4	10.0 ± 3.4	13.8 ± 3.4	11.5 ± 3.4	13.2 ± 3.4	12.3 ± 3.4
	u	12.2 ± 3.4	18.3 ± 3.4	12.0 ± 3.4	13.0 ± 3.4	16.0 ± 3.4	17.2 ± 3.4	11.7 ± 3.4	11.0 ± 3.4
Decapitated	v	2.0 ± 1.0	0.2 ± 1.0	0.7 ± 1.0	1.5 ± 1.0	1.0 ± 1.0	1.7 ± 1.0	2.7 ± 1.0	5.5 ± 1.0
	u	2.3 ± 1.0	0.3 ± 1.0	0.7 ± 1.0	2.8 ± 1.0	1.5 ± 1.0	2.2 ± 1.0	0.7 ± 1.0	1.0 ± 1.0
Flat	v	2.3 ± 0.7	2.0 ± 0.7	1.8 ± 0.7	3.5 ± 0.7	2.2 ± 0.7	2.3 ± 0.7	2.0 ± 0.7	2.6 ± 0.7
	u	2.7 ± 0.7	2.5 ± 0.7	1.2 ± 0.7	1.5 ± 0.7	2.7 ± 0.7	3.0 ± 0.7	2.8 ± 0.7	1.2 ± 0.7
Elongated	v	4.5 ± 1.3	4.0 ± 1.3	3.0 ± 1.3	7.3 ± 1.3	3.5 ± 1.3	5.7 ± 1.3	4.3 ± 1.3	3.8 ± 1.3
	u	1.3 ± 1.3	3.7 ± 1.3	4.2 ± 1.3	4.3 ± 1.3	4.8 ± 1.3	3.7 ± 1.3	4.5 ± 1.3	7.1 ± 1.3
Cratered	v	6.0 ± 1.0	2.5 ± 1.0	5.5 ± 1.0	2.7 ± 1.0	4.8 ± 1.0	2.7 ± 1.0	4.3 ± 1.0	4.8 ± 1.0
	u	2.8 ± 1.0	4.8 ± 1.0	7.2 ± 1.0	2.5 ± 1.0	3.5 ± 1.0	4.2 ± 1.0	4.0 ± 1.0	3.0 ± 1.0
Pyriform	v	1.2 ± 0.5	0.7 ± 0.5	0.8 ± 0.5	0.2 ± 0.5	1.2 ± 0.5	1.0 ± 0.5	0.2 ± 0.5	1.0 ± 0.5
	u	0.8 ± 0.5	1.8 ± 0.5	1.2 ± 0.5	1.0 ± 0.5	1.7 ± 0.5	2.0 ± 0.5	1.3 ± 0.5	1.7 ± 0.5
Asymmetric	v	0.5 ± 0.7	0.8 ± 0.7	0.7 ± 0.7	0.2 ± 0.7	1.3 ± 0.7	0.3 ± 0.7	0.3 ± 0.7	1.2 ± 0.7
	u	1.3 ± 0.7	2.4 ± 0.7	1.0 ± 0.7	0.5 ± 0.7	0.8 ± 0.7	2.0 ± 0.7	0.5 ± 0.7	1.3 ± 0.7
Short	v	0.3 ± 0.2	0.5 ± 0.2	0.0 ± 0.2	0.0 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.0 ± 0.2
	u	0.3 ± 0.2	0.2 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.2

[a]site v = vaginal semen deposition
u = intrauterine semen deposition

[b]Cervical region: Central: obtained by curettage of cervical mucosa proximal to cervical canal
Peripheral: obtained by vacuum deeper between 1° and 2° folds of cervical mucosa

[c]Cervical quarter = Qtr. numbered sequentially 1-4 from vaginal to uterine aspect

Appendix Table 21. Least squares means ± standard errors for percentages of sperm both alive and having head abnormalities in the cervix by insemination site[a], cervical region[b], and cervical quarter[c]. Experiment 2.

Sperm Trait	Site	Cervical Region							
		Central				Peripheral			
		Qtr1	Qtr2	Qtr3	Qtr4	Qtr1	Qtr2	Qtr3	Qtr4
Live Tapered	v	5.7 ± 2.9	7.3 ± 2.9	10.2 ± 2.9	6.5 ± 2.9	10.7 ± 2.9	8.5 ± 2.9	8.7 ± 2.9	9.4 ± 2.9
	u	7.8 ± 2.9	12.4 ± 2.9	5.2 ± 2.9	8.5 ± 2.9	9.6 ± 2.9	11.5 ± 2.9	7.3 ± 2.9	6.8 ± 2.9
Live Decapitated	v	0.7 ± 0.5	0.0 ± 0.5	0.2 ± 0.5	0.5 ± 0.5	0.5 ± 0.5^{x}	0.3 ± 0.5^{x}	0.8 ± 0.5	2.8 ± 0.5^{y}
	u	0.0 ± 0.5	0.0 ± 0.5	0.2 ± 0.5	1.0 ± 0.5	0.2 ± 0.5	0.5 ± 0.5	0.5 ± 0.5	0.0 ± 0.5
Live Flat	v	0.7 ± 0.6	1.2 ± 0.6	1.8 ± 0.6	1.7 ± 0.6	1.7 ± 0.6	1.5 ± 0.6	1.2 ± 0.6	2.3 ± 0.6
	u	1.5 ± 0.6	1.7 ± 0.6	0.7 ± 0.6	1.2 ± 0.6	1.5 ± 0.6	2.5 ± 0.6	1.8 ± 0.6	0.3 ± 0.6
Live Elongated	v	0.8 ± 0.7	1.3 ± 0.7	1.3 ± 0.7	3.8 ± 0.7	2.0 ± 0.7	2.7 ± 0.7	1.5 ± 0.7	1.8 ± 0.7
	u	0.3 ± 0.7	1.2 ± 0.7	1.7 ± 0.7	1.8 ± 0.7	2.2 ± 0.7	1.8 ± 0.7	2.7 ± 0.7	2.8 ± 0.7
Live Cratered	v	2.0 ± 0.7	0.2 ± 0.7	2.0 ± 0.7	1.7 ± 0.7	2.5 ± 0.7	1.0 ± 0.7	1.3 ± 0.7	3.3 ± 0.7
	u	1.7 ± 0.7	1.7 ± 0.7	3.2 ± 0.7	1.2 ± 0.7	2.3 ± 0.7	2.0 ± 0.7	1.7 ± 0.7	0.7 ± 0.7
Live Pyriform	v	0.8 ± 0.3	0.5 ± 0.3	0.7 ± 0.3	0.0 ± 0.3	0.5 ± 0.3	0.7 ± 0.3	0.0 ± 0.3	0.5 ± 0.3
	u	0.3 ± 0.3	1.0 ± 0.3	0.8 ± 0.3	0.3 ± 0.3	0.8 ± 0.3	1.2 ± 0.3	1.0 ± 0.3	1.0 ± 0.3
Live Asymmetric	v	0.0 ± 0.5	0.2 ± 0.5	0.3 ± 0.5	0.2 ± 0.5	0.8 ± 0.5	0.2 ± 0.5	0.3 ± 0.5	1.0 ± 0.5
	u	0.7 ± 0.5	1.7 ± 0.5	0.8 ± 0.5	0.3 ± 0.5	0.0 ± 0.5	0.2 ± 0.5	0.3 ± 0.5	0.2 ± 0.5
Live Short	v	0.2 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.0 ± 0.1	0.0 ± 0.1
	u	0.2 ± 0.1	0.2 ± 0.1	0.0 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1

[a]site v = vaginal semen deposition
u = intrauterine semen deposition

[b]Cervical region: Central: obtained by curettage of cervical mucosa proximal to cervical canal
Peripheral: obtained by vacuum deeper between 1° and 2° folds of cervical mucosa

[c]Cervical quarter = Qtr. numbered sequentially 1-4 from vaginal to uterine aspect

[x,y]Row means within cervical region having unlike superscripts differ ($p<.05$).

Appendix Table 22. Least squares means ± standard errors for percentages of sperm with head abnormalities in the cervix by cervical region[a] and cervical quarter[b] and pooled across insemination sites. Experiment 2.

Sperm Trait	Cervical Region							
	Central				Peripheral			
	Qtr1	Qtr2	Qtr3	Qtr4	Qtr1	Qtr2	Qtr3	Qtr4
Tapered	12.3 ± 2.4	16.0 ± 2.4	13.5 ± 2.4	11.5 ± 2.4	14.9 ± 2.4	14.3 ± 2.4	12.4 ± 2.4	11.6 ± 2.4
Decapitated	2.2 ± 0.7	0.3 ± 0.7	0.7 ± 0.7	2.2 ± 0.7	1.2 ± 0.7	1.9 ± 0.7	1.7 ± 0.7	3.2 ± 0.7
Flat	2.5 ± 0.5	2.3 ± 0.5	1.5 ± 0.5	2.5 ± 0.5	2.4 ± 0.5	2.7 ± 0.5	2.4 ± 0.5	1.9 ± 0.5
Elongated	2.9 ± 0.9	3.8 ± 0.9	3.6 ± 0.9	5.8 ± 0.9	4.2 ± 0.9	4.7 ± 0.9	4.4 ± 0.9	5.5 ± 0.9
Cratered	4.4 ± 0.7	3.7 ± 0.7	6.3 ± 0.7*	2.6 ± 0.7*	4.2 ± 0.7	3.4 ± 0.7	4.2 ± 0.7	3.9 ± 0.7
Pyriform	1.0 ± 0.4	1.3 ± 0.4	1.0 ± 0.4	0.6 ± 0.4	1.4 ± 0.4	1.5 ± 0.4	0.8 ± 0.4	1.3 ± 0.4
Asymmetric	0.9 ± 0.5	1.6 ± 0.5	0.8 ± 0.5	0.3 ± 0.5	1.1 ± 0.5	1.2 ± 0.5	0.4 ± 0.5	1.3 ± 0.5
Short	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
Live Tapered	6.7 ± 2.0	9.9 ± 2.0	7.7 ± 2.0	7.5 ± 2.0	10.1 ± 2.0	10.0 ± 2.0	8.0 ± 2.0	8.1 ± 2.0
Live Decapitated	0.3 ± 0.3	0.0 ± 0.3	0.2 ± 0.3	0.8 ± 0.3	0.3 ± 0.3	0.4 ± 0.3	0.7 ± 0.3	1.4 ± 0.3
Live Flat	1.1 ± 0.4	1.4 ± 0.4	1.3 ± 0.4	1.4 ± 0.4	1.6 ± 0.4	2.0 ± 0.4	1.5 ± 0.4	1.3 ± 0.4
Live Elongated	0.6 ± 0.5	1.3 ± 0.5	1.5 ± 0.5	2.8 ± 0.5	2.1 ± 0.5	2.3 ± 0.5	2.1 ± 0.5	2.3 ± 0.5
Live Cratered	1.8 ± 0.5	0.9 ± 0.5	2.6 ± 0.5	1.4 ± 0.5	2.4 ± 0.5	1.5 ± 0.5	1.5 ± 0.5	2.0 ± 0.5
Live Pyriform	0.6 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.2 ± 0.2	0.7 ± 0.2	0.9 ± 0.2	0.5 ± 0.2	0.8 ± 0.2
Live Asymmetric	0.3 ± 0.4	0.9 ± 0.4	0.6 ± 0.4	0.3 ± 0.4	0.7 ± 0.4	0.8 ± 0.4	0.3 ± 0.4	0.9 ± 0.4
Live Short	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1

[a]Cervical region: Central: obtained by curettage of cervical mucosa proximal to cervical canal
Peripheral: obtained by vacuum deeper between 1° and 2° folds of cervical mucosa

[b]Cervical Quarter: Qtr. numbered sequentially 1-4 from vaginal to uterine aspect

*means differ ($p<.05$)

Appendix Table 23. Least squares means ± standard errors for percentages of sperm with head abnormalities in the cervix by cervical region[a] and pooled across insemination sites and cervical quarters. Experiment 2.

Sperm Trait	Cervical Region	
	Central	Peripheral
Tapered	13.3 ± 1.8	13.3 ± 1.8
Decapitated	1.3 ± 0.4	2.0 ± 0.4
Flat	2.2 ± 0.4	2.4 ± 0.4
Elongated	4.0 ± 0.5	4.7 ± 0.5
Cratered	4.3 ± 0.2	3.9 ± 0.2
Pyriform	1.0 ± 0.2	1.3 ± 0.2
Asymmetric	0.9 ± 0.3	1.0 ± 0.3
Short	0.3 ± 0.1	0.2 ± 0.1
Live Tapered	7.9 ± 1.5	9.1 ± 1.5
Live Decapitated	0.3 ± 0.2	0.7 ± 0.2
Live Flat	1.3 ± 0.1	1.6 ± 0.1
Live Elongated	1.5 ± 0.4	2.2 ± 0.4
Live Cratered	1.7 ± 0.2	1.9 ± 0.2
Live Pyriform	0.6 ± 0.03	0.7 ± 0.03*
Live Asymmetric	0.5 ± 0.2	0.7 ± 0.2
Live Short	0.1 ± 0.04	0.1 ± 0.04

[a]Sample Location: Central: obtained by curettage of cervical mucosa proximal to cervical canal
Peripheral: obtained by vacuum deeper between 1^{o} and 2^{o} folds of cervical mucosa

*means within line differ ($p<.05$)

Appendix Table 24. Least squares means ± standard errors for percentages of sperm quality traits in the vaginal mucus of intrauterine inseminated heifers compared to those in other sample locations. Experiment 2.

Sperm Trait	Vaginal Mucus	Inseminate	Cervix		Uterus
			Central	Peripheral	
Tapered	11.3 ± 2.6	7.2 ± 2.6	13.8 ± 2.6	13.9 ± 2.6	9.2 ± 2.6
Decapitated	7.3 ± 6.1	12.3 ± 6.1	1.5 ± 6.1	1.3 ± 6.1	4.3 ± 6.1
Flat	4.2 ± 0.9	4.0 ± 0.9	2.0 ± 0.9	2.4 ± 0.9	3.3 ± 0.9
Elongated	1.5 ± 1.1	3.5 ± 1.1	3.4 ± 1.1	5.0 ± 1.1	3.3 ± 1.1
Cratered	3.2 ± 0.7	3.3 ± 0.7	4.3 ± 0.7	3.7 ± 0.7	3.5 ± 0.7
Pyriform	2.8 ± 0.8	1.3 ± 0.8	1.2 ± 0.8	1.7 ± 0.8	0.7 ± 0.8
Asymmetric	0.8 ± 0.4	0.5 ± 0.4	1.3 ± 0.4	1.2 ± 0.4	0.2 ± 0.4
Short	0.0 ± 0.2	0.5 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.0 ± 0.2
Live Tapered	3.2 ± 2.2	2.7 ± 2.2	8.5 ± 2.2	8.8 ± 2.2	7.5 ± 2.2
Live Decapitated	0.0 ± 0.2	0.8 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2
Live Flat	1.5 ± 0.8	2.0 ± 0.8	1.3 ± 0.8	1.5 ± 0.8	2.2 ± 0.8
Live Elongated	0.7 ± 0.7	1.5 ± 0.7	1.3 ± 0.7	2.4 ± 0.7	2.0 ± 0.7
Live Cratered	0.7 ± 0.3	1.2 ± 0.3	1.9 ± 0.3*	1.7 ± 0.3	1.8 ± 0.3
Live Pyriform	1.5 ± 0.5	0.7 ± 0.5	0.6 ± 0.5	1.0 ± 0.5	0.5 ± 0.5
Live Asymmetric	0.3 ± 0.3	0.3 ± 0.3	0.9 ± 0.3	0.7 ± 0.3	0.2 ± 0.3
Live Short	0.0 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.0 ± 0.2

*Differs from vaginal mucus mean ($p<.05$).

Appendix Table 25. Least squares means ± standard errors for percentages of sperm head abnormalities in the retrograde mucus sperm population compared to those in other sample locations by insemination site.[a] Experiment 2.

Sperm Trait	Site	Retrograde Mucus	Inseminate	Cervix		Uterus
				Central	Peripheral	
Tapered	v	11.6 ± 2.4	7.2 ± 2.4	12.8 ± 2.4	12.7 ± 2.4	16.0 ± 2.4
	u	9.9 ± 3.0	7.2 ± 2.4	13.9 ± 2.4	13.9 ± 2.4	9.2 ± 2.4
Decapitated	v	10.0 ± 5.8	12.3 ± 5.8	1.1 ± 5.8	2.7 ± 5.8	1.0 ± 5.8
	u	22.8 ± 7.4	12.3 ± 5.8	1.5 ± 5.8	1.3 ± 5.8	4.3 ± 5.8
Flat	v	6.5 ± 1.0	4.0 ± 1.0	2.4 ± 1.0	2.3 ± 1.0	4.8 ± 1.0
	u	5.4 ± 1.2	4.0 ± 1.0	2.0 ± 1.0	2.4 ± 1.0	3.3 ± 1.0
Elongated	v	2.2 ± 1.2	3.5 ± 1.2	4.7 ± 1.2	4.3 ± 1.2	4.2 ± 1.2
	u	1.1 ± 1.5	3.5 ± 1.2	3.4 ± 1.2	5.0 ± 1.2	3.3 ± 1.2
Cratered	v	2.2 ± 0.6	3.3 ± 0.6	4.2 ± 0.6	4.2 ± 0.6	2.8 ± 0.6
	u	1.0 ± 0.8	3.3 ± 0.6	4.3 ± 0.6	3.7 ± 0.6	3.5 ± 0.6
Pyriform	v	2.2 ± 0.3	1.3 ± 0.3	0.7 ± 0.3*	0.8 ± 0.3*	1.0 ± 0.3*
	u	0.5 ± 0.3	1.3 ± 0.3	1.2 ± 0.3	1.7 ± 0.3	0.7 ± 0.3
Asymmetric	v	0.5 ± 0.8	0.5 ± 0.8	0.5 ± 0.8	0.8 ± 0.8	2.8 ± 0.8
	u	0.1 ± 1.0	0.5 ± 0.8	1.3 ± 0.8	1.2 ± 0.8	0.2 ± 0.8
Short	v	0.5 ± 0.3	0.5 ± 0.3	0.2 ± 0.3	0.1 ± 0.3	0.2 ± 0.3
	u	0.5 ± 0.3	0.5 ± 0.3	0.3 ± 0.3	0.3 ± 0.3	0.0 ± 0.3

[a]Insemination site v = vaginal semen deposition
u = intrauterine semen deposition

*Differs from retrograde mucus mean ($p<.05$)

Appendix Table 26. Least squares means ± standard errors for percentages of sperm both alive and having head abnormalities in the retrograde mucus sperm population compared to those in other sample locations by insemination site.[a] Experiment 2.

Sperm Trait	Site	Retrograde Mucus	Inseminate	Cervix		Uterus
				Central	Peripheral	
Live Tapered	v	9.2 ± 2.4	2.7 ± 2.4	7.4 ± 2.4	9.3 ± 2.4	14.0 ± 2.4
	u	2.7 ± 3.1	2.7 ± 2.4	8.5 ± 2.4	8.8 ± 2.4	7.5 ± 2.4
Live Decapitated	v	1.5 ± 0.4	0.8 ± 0.4	0.3 ± 0.4	1.1 ± 0.4	0.0 ± 0.4
	u	0.2 ± 0.6	0.8 ± 0.4	0.3 ± 0.4	0.3 ± 0.4	0.3 ± 0.4
Live Flat	v	5.3 ± 0.8	2.0 ± 0.8	1.3 ± 0.8	1.7 ± 0.8	4.5 ± 0.8
	u	1.2 ± 1.0	2.0 ± 0.8	1.3 ± 0.8	1.5 ± 0.8	2.2 ± 0.8
Live Elongated	v	1.3 ± 0.8	1.5 ± 0.8	1.8 ± 0.8	2.0 ± 0.8	3.3 ± 0.8
	u	0.6 ± 1.1	1.5 ± 0.8	1.3 ± 0.8	2.4 ± 0.8	2.0 ± 0.8
Live Cratered	v	1.7 ± 0.4	1.2 ± 0.4	1.5 ± 0.4	2.0 ± 0.4	2.0 ± 0.4
	u	0.5 ± 0.5	1.2 ± 0.4	1.9 ± 0.4	1.7 ± 0.4	1.8 ± 0.4
Live Pyriform	v	1.7 ± 0.2	0.7 ± 0.2	0.5 ± 0.2*	0.7 ± 0.2	0.7 ± 0.2
	u	0.4 ± 0.3+	0.7 ± 0.2	0.6 ± 0.2	1.0 ± 0.2	0.5 ± 0.2
Live Asymmetric	v	0.5 ± 0.7	0.3 ± 0.7	0.2 ± 0.7	0.6 ± 0.7	2.8 ± 0.7
	u	0.1 ± 0.9	0.3 ± 0.7	0.9 ± 0.7	0.7 ± 0.7	0.2 ± 0.7
Live Short	v	0.3 ± 0.2	0.3 ± 0.2	0.0 ± 0.2	0.1 ± 0.2	0.0 ± 0.2
	u	0.2 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.0 ± 0.2

[a]Insemination site v = vaginal semen deposition
u = intrauterine semen deposition

*Differs from retrograde mucus mean ($p<.05$)

+Column means within sperm trait differ ($p<.05$)

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