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Heterospermic Insemination in Swine

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HETEROSPERMIC INSEMINATION IN SWINE

BY

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HETEROSPERMIC INSEMINATION IN SWINE

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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HETEROSPERMIC INSEMINATION IN SWINE

Abstract

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Under the supervision of Associate Professor A. L. Slyter

The studies reported herein were conducted to evaluate (1) if there is a difference in pregnancy rate and litter size in swine between heterospermic and homospermic inseminations, (2) the effect of mixing various fresh semen components involving spermatozoa in the sperm-rich fraction, seminal plasma from the centrifuged post-sperm fraction and Beltsville Ll extender on oxygen consumption, pH, motility and percent dead spermatozoa and (3) the effect of storage (54 hours) on oxygen consumption, pH, motility and percent dead spermatozoa in the various combinations. One Hampshire and one Yorkshire boar were used in the pregnancy rate and litter size study.

Pregnancy rate results indicated no difference ($P > .05$) between gilts inseminated with mixed semen (50%), Yorkshire semen (43%) or Hampshire semen (33%). Gilts were slaughtered at a mean gestation length of 33.7 (trial I) and 42.2 (trial II) days. The mean numbers of embryos present at time of slaughter were 9.8, 6.4 and 5.7 for the gilts bred with mixed semen, Hampshire semen and Yorkshire semen, respectively.

When studying oxygen consumption, pH, motility and percent dead spermatozoa in the laboratory, two boars were used (one Hampshire and one Yorkshire). All 21 combinations were represented on the Warburg at one time.

Analysis of total microliters of oxygen consumed showed differences ($P < .005$) for treatments, fresh vs stored and days. However, definite conclusions could not be drawn due to interactions of treatment x fresh vs stored ($P < .005$), treatment x day ($P < .005$) and fresh vs stored x day ($P < .05$). A quadratic ($P < .05$) polynomial regression for accumulative microliters of oxygen consumed was found for fresh semen samples, whereas a linear relationship was observed in stored samples. A cubic ($P < .05$) regression line was found in stored semen and a linear in fresh samples for microliters of oxygen taken up per interval.

When Hampshire sperm-rich, Yorkshire sperm-rich and mixed sperm-rich were diluted with centrifuged Yorkshire post-sperm plasma as compared to centrifuged Hampshire post-sperm plasma, higher ($P < .005$) pH values and lower ($P < .05$) motility estimates were found in fresh and stored samples. These differences were present before and after incubation in the Warburg. Motility estimates (stored samples) for Hampshire sperm-rich, Yorkshire sperm-rich and mixed sperm-rich diluted with Hampshire post-sperm plasma were 49.4, 48.0 and 48.1%, respectively, after incubation in the Warburg and essentially zero when warmed in a 38 C water bath. Zero motility was recorded before and after incubation for Hampshire sperm-rich, Yorkshire sperm-rich and mixed sperm-rich diluted with Yorkshire post-sperm plasma.

A greater ($P < .05$) number of dead spermatozoa was recorded when Yorkshire post-sperm plasma was used by itself as a diluent for Hampshire sperm-rich, Yorkshire sperm-rich and mixed sperm-rich.

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LIST OF ABBREVIATIONS AND SYMBOLS

BL1 . . . . . Beltsville Ll extender

CPS . . . . . Centrifuged post-sperm

Conc . . . . Sperm concentration

g . . . . . Gravity, centrifuged

PS . . . . . Post-sperm

PSP . . . . . Post-sperm plasma

SR . . . . . Sperm-rich

MS . . . . . Mixed sperm

HS . . . . . Hampshire sperm

HPS . . . . . Hampshire post-sperm

HPSP . . . . Hampshire post-sperm plasma

HSR . . . . . Hampshire sperm-rich

IU . . . . . International Unit

µl . . . . . Microliter(s)

$\mu l O_{2I}$ . . . . Microliters of oxygen uptake per 10^8 sperm cells per interval

$\mu l O_{2A}$ . . . . Microliters of oxygen accumulated per 10^8 sperm cells during the incubation period

YS . . . . . Yorkshire sperm

YPS . . . . . Yorkshire post-sperm

YPSP . . . . Yorkshire post-sperm plasma

YSR . . . . . Yorkshire sperm-rich

ns . . . . . Nonsignificant

INTRODUCTION

The artificial insemination industry should be dedicated to provide semen from genetically superior sires which results in the highest possible conception rate.

Russian investigations have indicated that heterospermic insemination in cattle, sheep and swine is considerably better than that of homospermic controls. Characteristics claimed to be enhanced are conception rate, litter size, birth weight, growth rate and viability of progeny. However, many of the Russian experiments are open to criticism because of experimental procedure and subjective measurements employed.

It has been theorized that mixing semen may produce a higher conception rate and litter size due to interactions of (1) spermatozoa and spermatozoa, (2) spermatozoa and seminal plasma, (3) seminal plasma and seminal plasma, (4) spermatozoa and the female reproductive tract and (5) seminal plasma and the female reproductive tract.

Another consideration is that mixing semen may increase the number of sperm maturation stages and thus increase the chances of having optimally mature spermatozoa present at ovulation.

The objectives of this investigation were to (1) determine if there is a difference in conception rate and litter size in swine between heterospermic and homospermic inseminations, (2) study the effect of mixing various semen components involving spermatozoa in the sperm-rich fraction, seminal plasma from the centrifuged post-sperm fraction and Beltsville Ll extender on oxygen consumption, pH,

motility and percent dead spermatozoa and (3) study the effect of storage on oxygen consumption, pH, motility and percent dead spermatozoa in the various combinations.

REVIEW OF LITERATURE

Boar semen characteristics of value for research investigations are (1) a large volume of sample to work with, (2) the ejaculate can be visually fractionated into pre-sperm, sperm-rich, post-sperm and gelatinous fractions, (3) spermatozoa and seminal plasma are easily obtained and readily replaced, (4) spermatozoa are normally detached from each other which results in gases being able to diffuse without passing through connective tissue and (5) spermatozoa do not undergo multiplication or cell division.

Principal Features of a Mature Spermatozoon

Mammalian spermatozoa vary greatly in their microscopic appearance according to the species (Fawcett and Phillips, 1970). Boar spermatozoon consists of a head, middle piece and tail in a filiform structure. According to Hancock (1956) the dimensions of an average boar spermatozoon is 8.5 µm long by 4.2 µm wide for the head, 10 µm for the middle piece and 30 µm for the tail. The shape of the head of a normal spermatozoon of a bull, boar, ram, rabbit and man is oval, elongated cylinder in fowl and hook-like in the mouse, rat and guinea pig (Phillips, 1974; Fawcett and Phillips, 1970). The sperm nucleus and acrosome are the main components of the head. The predominant constituent of the nucleus is deoxyribonucleic acid, whereas the acrosome is composed of a lipoglycoprotein and enzyme complex (Mann, 1967). The middle piece is surrounded by a mitochondrial sheath which is composed of a set of enzymes and cytochrome system necessary for

glycolysis and respiration (Balogh and Cohen, 1964; Fleeger and Flipse, 1964; Flipse, 1964; Mann, 1964; Semakov, 1964; Stallcup and Roussel, 1964; White, 1958).

The main biological function of boar spermatozoa is twofold: to initiate development of the ova and to transfer paternal genetic material.

Effect of Mixing Ejaculates From Different Males

Sheep

It appears from the literature that the original investigations on the effects of heterospermic insemination were performed in Russia. In 1946, 1947 and 1948 Abuljhanov (1950) studied the effect of inseminating ewes with mixed semen. The experiment was composed of 122 experimental ewes and 90 control ewes. Analysis of the data revealed that all experimental ewes lambed and only 85 of the control ewes lambed. The ewes inseminated with mixed semen produced an average of 138 (130 to 147) lambs per 100 ewes, whereas the ewes inseminated with unmixed semen produced an average of 102 (100 to 105) lambs per 100 ewes. This indicates that more twins were born in the experimental group than in the control group of ewes. Within the experimental group there was a difference in lambing rate between those ewes inseminated with mixed semen from rams of a different breed (138 to 147 lambs per 100 ewes) than those ewes inseminated with mixed semen from rams of the same breed (130 lambs per 100 ewes).

Cattle

Several investigations have been conducted in studying the effect of heterospermic semen on conception rate in cattle. The majority of the literature indicates an enhanced effect on conception rate. Russian work (Radnabazaron, 1951; Klyuchnikov, 1970) has shown an enhanced effect on conception rate of 8.0 to 20.2% for heterospermic insemination. An 8 to 11% increase in conception rate has been reported by Mijavec (1961) and Keetch and Butcher (1969) in favor of heterospermic semen.

This enhanced response in conception rate due to heterospermic semen has been reported by Hess (1953) and co-workers (1954, 1958). These investigators found Holstein (H) and Guernsey (G) heterospermic sperm (three different bulls within a breed) had a higher ($P < .01$) first service nonreturn rate than homospermic semen (H, 68.4 vs 57.0; G, 69.6 vs 61.6%). Stewart et al. (1974) mixed semen from four Holstein bulls and reported a nonreturn rate at 4 and 16 weeks for the heterospermic and homospermic groups of 86.4% and 71.9% vs 79% and 63%, respectively. Heterospermic semen (three different bulls within a breed) produced a higher ($P < .05$) nonreturn rate than homospermic semen (6.3 and 4.5%) in both Angus and Polled Hereford breeds, respectively (Elliott, 1974).

First service pregnancy rate for heterospermic semen (70.6%) containing three bulls' semen was higher ($P < .05$) than homospermic semen (62.7%) or heterospermic semen (62.2%) prepared from two bulls (Nelson et al., 1975). Heterospermic mixtures in liquid form of two bulls produced a higher (68.1 vs 65.5%) nonreturn rate at 16 weeks than

individual bulls (Beatty et al., 1969). In contrast to results reported by Beatty et al. (1969), Nelson et al. (1975) found the combination of two bulls' semen in one mixture resulted in a 7.7% lower pregnancy rate in cows than the homospermic semen on first service. When heifers were bred with semen from the same bulls as used on the cows, Nelson et al. (1975) observed a 9.8% increase in pregnancy rate for the heterospermic mixture on first service.

Frappell and Williams (1956) have stated there is no difference in conception rate between first service inseminations with heterospermic or homospermic semen. They used five pairs of Hereford bulls with each pair consisting of two bulls having similar previous conception rates. During a 4-month period conception rates for three of the pairs were higher for homospermic semen by 4.5%, 3.4% and 2.0%, respectively, while for the other two pairs there were differences of .1% and .5% in favor of heterospermic semen. When the data were summed and averaged for all pairs over the 4-month period, the homospermic semen gave a conception rate 2.2% higher than heterospermic semen. Zelfel and Gottschalk (1962) have shown similar results on conception rate between heterospermic (43.8%) and homospermic (43.6%) inseminations.

The effect of heterospermic semen on second service conception rate has also been studied (Hess et al., 1958; Elliott, 1974; Nelson et al., 1975). Hess et al. (1958) found heterospermic semen to perform 11.6% better ($P < .01$) on repeat services than homospermic semen. Elliott (1974) did not find a benefit for heterospermic semen on second service

inseminations (54.1 vs 50.0%). Nelson et al. (1975) reported the second service pregnancy rate of homospermic semen was 2.8% higher than heterospermic semen (two bulls). An increase ($P > .10$) in pregnancy rate was found for cows (2.1%) and heifers (2.9%) when bred with heterospermic semen (three bulls) as compared to homospermic semen in the second service (Nelson et al., 1975). Pregnancy rates in cows bred with heterospermic semen (78%) from three bulls for first and second services combined over years were higher than the homospermic (73.4%, $P > .05$) and heterospermic semen (69.7%, $P < .05$) from two bulls. When first and second services were combined for analyzing percent heifers pregnant, a slight increase ($P > .05$) for heterospermic inseminations (two bulls, 78.9%; three bulls, 80.0%) was found over homospermic inseminations (72.1%).

Research data have been examined to specifically find whether the conception rate of heterospermic semen is superior to each individual contributing to the mixture. Heterospermic (Simmental + Friesian) conception rate (72.6%) reported by Mijavec (1961) was better than either of the individual bulls contributing to the mixture (Simmental, 53.5%; Friesian, 57.4%). Conception rate for heterospermic semen (Hereford + Shorthorn) was 51% as compared to homospermic conception rates of 40% for Hereford and 47% for Shorthorn (Keetch and Butcher, 1969). Cows bred with mixed semen from three Angus bulls had a higher ($P < .05$) conception rate than cows bred with homospermic semen from the three bulls (Elliott, 1974). Elliott (1974) reported identical results for the combination of three Polled Hereford bulls. Nelson

et al. (1975) reported the pregnancy rate of each heterospermic (two or three bulls) treatment for first service was lower than the pregnancy rate of the highest individual bull in the mixture in all but two of 15 comparisons. Heterospermic semen (two or three bulls) did produce a higher pregnancy rate than the lowest individual bull for first service. Second service data showed that heterospermic semen (two or three bulls) produced a lower pregnancy rate than the lowest individual bull in two of 15 comparisons.

The hypothesis that an equal number of progeny should result from each sire contributing to the mixture has been investigated (Radnabazaron, 1951; Stewart et al., 1974; Elliott, 1974; Nelson et al., 1975). When Astrakhan cows were bred with a mixture of Astrakhan, Hereford and Shorthorn semen, the progeny results were nine Astrakhan, three crossbred Hereford and three crossbred Shorthorn. When four breeds of bulls were combined into a mixture for breeding Astrakhan cows, four Astrakhan, one crossbred Hereford, two crossbred Shorthorn and no crossbred Angus calves were born (Radnabazaron, 1951). Fertility differences between three different bulls (A, B and C) in a mixture have been reported by Elliott (1974). Bulls A, B and C sired 26, 63 and 11%, respectively, of the total calves born.

When cows were inseminated with fresh (unfrozen) mixtures of semen from four bulls, Stewart et al. (1974) found no difference from a theoretical ratio of 1:1:1:1 for the number of progeny sired by each bull. When semen from the same four bulls was mixed and frozen, the number of progeny per bull differed ($P < .001$) from the 1:1:1:1 ratio.

The difference between the fresh and frozen semen was due to one bull producing 23% of the progeny in the fresh group and 45% in the frozen group. The ability of bull semen to withstand deep freezing and thawing has been shown to vary between bulls (Robbins et al., 1972; Saacke and White, 1972; Wiggins and Almquist, 1975).

Nelson et al. (1975), using frozen semen from three bulls (A, B and C), found that bull A sired the greatest number of progeny in any mixture containing his semen. The combinations AB, AC and ABC showed bull A sired 76.9, 70.6 and 47.2%, respectively, of the total calves born. The combination BC resulted in bull B producing 58.3% of the calves.

Swine

Literature concerning the use of mixed semen in swine is very limited, of Russian origin and in abstract form.

Hlebov (1965) observed a difference in conception rate for gilts inseminated with heterospermic semen (two or more boars) as compared to those bred with homospermic semen (83.3% vs 71.4%). Vasiljev et al. (1968) reported a 15.2% advantage in conception rate for heterospermic semen. Popenko (1969) mated 63 Large White (LW) sows with semen mixtures of two LW boars, one LW plus one Estonian (E) or one each from LW, E and Landrace breeds. In the three groups the conception rate averaged 61.9, 79.2 and 88.8%, respectively. Mixed boar semen from three breeds, Russian Large White (RLW), Landrace (L), Ukrainian Spotted Steppe (USS), was investigated by Minin et al. (1970). Seventy gilts were inseminated with RLW, L, USS, RLW + L, RLW + USS or RLW + USS + L semen and

conception rates were 81.2, 100.0, 77.7, 83.3, 92.3 and 93.3%, respectively. Heterospermic semen produced a higher conception rate than homospermic RLW and USS but not homospermic L. Cerne and Salehar (1964) reported no difference ($P > .05$) between heterospermic and homospermic semen for percent of sows farrowing.

Litter size has been of concern in heterospermic semen research. Heterospermic semen has produced a lower litter size (Hlebov, 1965, 7.9 vs 8.8; Vasiljev et al., 1968, 9.6 vs 9.9; Cerne and Salehar, 1964, 9.8 vs 10.9 pigs) than homospermic semen. Minin et al. (1970) found litter size to be 8.3, 11.5, 10.4, 11.2, 9.7 and 10.6 pigs for RLW, L, USS, RLW + L, RLW + USS and RLW + USS + L, respectively. When observing these data more closely, it is revealed that heterospermic semen (RLW + L and RLW + USS) produced a larger litter than the lowest litter size boar but not greater than the highest litter size boar in the combination. The combination RLW + USS + L produced a litter size close to the average of the homospermic controls (10.6 vs 10.1 pigs).

Rymarj (1963) studied the effect of mixed semen on the resemblance of progeny to sire. Seventy percent of the progeny resembled Landrace when Landrace and Large White boars were used. When mixed semen of Landrace, Large White and Ievlev boars was used, 60% of the progeny resembled Ievlev, 20% Landrace and 16% Large White. Evidently, 4% must not have been able to be positively identified.

Rabbits

Beatty (1957) observed the birth rate ($P > .05$) of litters born after heterospermic insemination (83.9%) was intermediate between the

birth rates of the homospermic controls (96.6 and 76.5%). In a later study Beatty (1960) found that when a Flemish Giant (FG) buck was used by himself for artificial insemination no litters were produced. A 52% conception rate was obtained when does were inseminated with semen composed of FG and an indeterminate breed (I) of buck. Inseminating does with FG + I + Netherland Dwarf gave a conception rate of 100%. The spermatozoa from FG were not incapable of fertilization because progeny were produced from FG in the mixed inseminates. In addition, no difference ($P > .05$) was revealed for conception rate between FG and I when used naturally.

Conception rate of does after heterospermic insemination (88%) reported by Napier (1961) was not different ($P > .05$) from the homospermic conception rate (75%). In three experiments conducted by Pickett et al. (1974) the mean kindling rate per doe bred with heterospermic semen (two or three males) was higher ($P > .05$) than homospermic inseminations, except for the kindling rate with heterospermic semen of three bucks in one experiment. Pickett et al. (1974) found heterospermic semen containing spermatozoa from four bucks produced a lower ($P < .05$) pregnancy rate than semen containing spermatozoa from two males. This does not agree with the suggestion that fertility of rabbits may increase as the number of males contributing to the inseminate increases (Beatty et al., 1969).

The number of progeny sired per buck varies from the theoretical 1:1 ratio. When the volume of heterospermic semen from two males was prepared by Beatty (1957) in a 50:50 ratio (H:C), male H sired 79.9%

of the progeny. Equal numbers of progeny were born when the mixture contained approximately 70% C and 30% H. This may not be a true representation of the two males because spermatozoa concentration per inseminate was not considered. Beatty (1960), in a later study, found the progeny ratio ($P < .005$) between two sires to be 1:5.

Pickett et al. (1974) found the number of progeny per doe for inseminates containing semen from one, two, three or four bucks was 2.1, 3.2, 3.5 and 2.0, respectively. The number of progeny per doe inseminated with heterospermic semen of four bucks was lower ($P < .10$) than that for heterospermic mixture of three bucks.

Chemical Components of Seminal Plasma and Spermatozoa

The effect of seminal plasma on spermatozoa metabolism may be better understood when complete information is available on the biochemical nature of seminal plasma and spermatozoa. Seminal plasma is a composite mixture of secretions which comes from the male accessory organs of reproduction.

Amino Acids and Proteins

The major amino acid revealed in porcine spermatozoa is glutamic acid. The second most prevalent is serine (Hood et al., 1967). These authors found a total of 20 different amino acids in the spermatozoa. Amino acids in spermatozoa among boars and ejaculates within boars were not different ($P > .10$).

The complete amino acid sequence of the basic nuclear protein of bull spermatozoa has been reported by Coelingh et al. (1972). These

investigators identified 47 amino acid residues with alanine at the amino terminus and glutamine at the carboxyl terminus. Twenty-four arginine residues were found in the protein.

The protein spectrum of boar seminal plasma has been investigated in recent years (Boursnell and Nelson, 1964; Boursnell et al., 1966, 1968a,b; Nelson and Boursnell, 1966; Boursnell and Briggs, 1969; Schellpfeffer and Hunter, 1970; Lavon and Boursnell, 1971; Lavon, 1972; Dostal and Veselsky, 1972; Lavon et al., 1973). These investigations have shown the majority of the seminal plasma proteins originate in the seminal vesicles and the quantitative contribution of the epididymides and prostate to the protein pattern is small. Twenty different proteins have been isolated with the majority of them being basic in character. The basic proteins comprise approximately 80 to 90% of the total amount of protein in seminal plasma. In contrast the seminal plasma of other mammals contains mixtures of acidic and neutral as well as basic proteins (Bennett, 1965; Lavon, 1972).

Johnson et al. (1969c) found 22 different amino acids in seminal plasma. Glutamic acid, taurine and glycine accounted for 74% and 87% of all amino acids in whole semen and the sperm-rich fraction, respectively. There was more ($P < .01$) glutamic acid in the sperm-rich fraction as compared to the whole ejaculate.

The free amino acid ergothioneine is also found in seminal vesicle secretions (Mann, 1964). Ergothioneine may function to protect spermatozoa by preventing the oxidation of sulfhydryl groups by

hydrogen peroxide and other inhibitors (Glover and Mann, 1954; Mann and Leone, 1953).

Lavon and Boursnell (1971) demonstrated that differences in the protein pattern of seminal plasma do exist between individual boars. The importance and significance between boars are not known.

Similar results for seminal vesicle secretions of free amino acids have been reported for the bull and ram (Brown et al., 1972).

Lipids

The fatty acid composition of boar spermatozoa and seminal plasma phospholipid has to some extent been elucidated (Johnson et al., 1969a,b,c, 1967; Grogan et al., 1966). Johnson and co-workers (1967, 1969a,b) studied the fatty acid composition of phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine in boar spermatozoa. The major saturated fatty acid in phosphatidyl choline and phosphatidyl ethanolamine was palmitic acid. However, stearic acid was the major saturated fatty acid in phosphatidyl serine. Unsaturated fatty acids found in boar spermatozoa accounted for 72.8 and 64.1% of total fatty acids in phosphatidyl choline and phosphatidyl ethanolamine, respectively. Docosapentaenoic acid was the predominating unsaturated fatty acid. Docosapentaenoic and docosahexaenoic acids represented 52 to 64% of the total fatty acid complement of both phosphatidyl choline and phosphatidyl serine.

The average total lipid content of boar spermatozoa (12%) was the same as that found in bovine spermatozoa (12%) but boar spermatozoa

was slightly higher in phospholipid and lower in cholesterol percentages (Komarek et al., 1965; Komarek, 1964).

Sphingomyelin was the predominate phospholipid found in boar seminal plasma (Johnson et al., 1969b). Komarek et al. (1965) found the lipid composition and percentage of total lipids in boar seminal plasma to be different between boars. Lipid content in boar seminal plasma was less than one-sixth that found in bull seminal plasma. The role of fatty acids in boar spermatozoa maturation or metabolism is unknown, but it seems likely that the highly unsaturated acids may be of importance.

Carbohydrates and Metabolic Products

The carbohydrates and/or derivatives that have been documented to be present in boar seminal plasma are fructose, galactose, glucose and mannose (McIntosh and Boursnell, 1966; Rottenberg and Boursnell, 1966; Mann, 1954, 1964). Various authors have indicated a low content of fructose (6.5 to 50.8 mg/100 ml) in boar seminal plasma (Aalbers et al., 1961; Foley et al., 1964; Mann, 1946, 1964; Schul, 1965).

Boar seminal plasma contains at least two sugar alcohols, sorbitol and inositol. King and Mann (1959) found a lower level of sorbitol in boar seminal plasma (6 to 18 mg/100 ml) than in ram (26 to 120 mg/100 ml), bull (10 to 36 mg/100 ml) and stallion (20 to 60 mg/100 ml) seminal plasma. Sorbitol dehydrogenase activity is low in boar seminal plasma which results in less sorbitol being oxidized to fructose (Aalbers et al., 1961).

Boar seminal vesicle secretions have been shown to be among the richest sources of unbound inositol in nature (Mann, 1964). Seminal inositol occurs in many species, but the level is generally 10 to 40 times lower than in the boar. Mann (1954) suggests that inositol is concerned with osmotic equilibrium maintenance since boar seminal plasma is low in sodium chloride.

Metabolic studies have shown that spermatozoa do not use citric acid as an energy source, although there is a high level of citric acid (45 to 700 mg/100 ml) in boar seminal plasma (Humphrey and Mann, 1949; White, 1958; Mann, 1964). Citric acid has been found to be secreted by the seminal vesicles (Davies et al., 1975). The role of citric acid may be in the formation of the gelatinous plug (White, 1958) or in combination with potassium and sodium ions to maintain osmotic equilibrium in semen (Mann, 1954). Boursnell et al. (1970) suggested that protein fractions A and H are the agents in vesicular secretions that promote gelatinous formation. These two proteins were found to be basic in nature by gel disc electrophoresis. Proteins A and H have isoelectric points of 8.8 and 9.4, respectively. Recently Boursnell and Butler (1973) found that protein H is incorporated into the gelatinous structure. Seminal vesiculectomized boars provided a seminal fluid which was more watery and did not form the characteristic gelatinous material after ejaculation. However, a sticky secretion was ejaculated and formed the characteristic gel when mixed with normal seminal plasma (Davies et al., 1975). This indicates that both the secretions from the bulbourethral gland and seminal vesicles are required for gelatinous formation.

Lactic acid, a readily oxidizable end-product of glycolysis for spermatozoa, is a normal constituent of seminal plasma (Mann, 1964; Wales and Wallace, 1965). Twenty-one mg of lactic acid per 100 ml of boar seminal plasma have been reported by Mann (1964).

Inorganic Elements

Electrolytes present in seminal plasma include calcium, chloride, phosphate, potassium and magnesium. In boar seminal plasma the ionic equilibrium on the cationic side is set up chiefly by potassium (212 mg/100 ml) with citric acid (635 mg/100 ml) as the main anion. The concentration of sodium (62 mg/100 ml), phosphorus (34 mg/100 ml) and chloride (12 mg/100 ml) is substantially lower than potassium (Mann, 1964). A high level of zinc (137 mg/100 ml) has been shown to be present in boar seminal vesicle secretions and to be correlated ($P < .01$) with citrate and total nitrogen in seminal fluid (Boursnell et al., 1972, 1973a,b; Boursnell and Roberts, 1974; Lavon and Boursnell, 1975). Published values for zinc content in seminal plasma differ between bull (< 2 µg/ml), ram (2.8 µg/ml), boar (22.5 µg/ml), man (134 µg/ml) and dog (71.3 to 86.5 µg/ml) (Boursnell et al., 1972; Cragle et al., 1958; Mann, 1964; Mawson and Fischer, 1953; Bartlett, 1962). Zinc is derived from the prostate gland in dog and man (Mawson and Fischer, 1951).

Enzymes

Mammalian seminal plasma contains a wide spectrum of very active enzymes. Some of the catalytic enzymes include phosphokinases, phospholipases, phosphatases, proteases, nucleases and glycosidases

(Mann, 1964, 1967). Enzyme determination in seminal plasma and spermatozoa has been mainly performed in human and bovine samples (Peterson and Freund, 1970a,b).

MATERIALS AND METHODS

Experiment 1 (Trials I and II)

The objective of this experiment was to study the effect of homospermic versus heterospermic insemination on the conception rate in gilts.

Management of Boars

Two boars (one Yorkshire and one Hampshire) of unknown fertility and trained to mount a dummy sow were used in trials I and II. Each boar was housed individually in the South Dakota State University boar barn and had free access to a small concrete exercise area outside of their shelter. The boars were hand fed approximately 2.5 kg per day of a 12% crude protein ration (table 1). This amount of feed kept the boars in a healthy and vigorous condition without allowing them to become excessively fat.

The approximate age of the boars at the beginning of trial I was 8 months for the Yorkshire and 14 months for the Hampshire. The boars were 12 and 18 months old, respectively, at the beginning of trial II.

Whole semen ejaculates were collected by the gloved-hand technique (Herrick and Self, 1962). The gelatinous portion was removed at the time of collection by a double layer of cheese cloth. All collections were received into thermos bottles which had been previously warmed to 38 C. After collecting the last boar, the semen

TABLE 1. RATION COMPOSITION FED BOARS AND GILTS DURING THE EXPERIMENTAL PERIOD

Nutrient	Percent of diet
Ground yellow corn	69.0
Ground oats	10.0
Dehydrated alfalfa meal (17%)	10.0
Soybean meal (44%)	7.0
Dicalcium phosphate	3.0
Trace mineral salt, .8% zinc	.5
Vitamin premix[a]	.5

[a] Provided per kg of diet: vitamin A, 4409 IU; vitamin D, 440.9 IU; riboflavin, 2.76 mg; pantothenic acid, 11.00 mg; niacin, 22.0 mg; choline, 110 mg and vitamin B_{12}, 16.53 mg.

was transported to a nearby laboratory and placed in a 38 C water bath until further processing.

Both boars were collected each day that semen was required to eliminate differences in collection frequencies between boars, even though only one gilt was in estrus and was to be bred with homospermic semen. The boars were collected 14 of 23 and 15 of 21 days during the experimental periods for trials I and II, respectively.

Semen Evaluation

Motility of the spermatozoa was estimated subjectively by placing a drop of semen on a glass coverslip and inverting the coverslip over a concave microscope slide to form a hanging drop suspension. Motility was estimated on a scale of 0 to 100% in 5% intervals while being observed under the low power magnification (100X) of a light microscope.

Sperm morphology and live-dead classifications were made once per week under the high power magnification (430X) of a light microscope. Rose-Bengal and nirgosine Eosin B stains (appendix A) were used for morphology and live-dead classifications, respectively. Ten cells in 10 different fields were counted and expressed as a percentage of the total.

Sperm concentration was estimated by a turbidometric method described by Young et al. (1960). All sperm concentrations used in trials I and II were determined by this method. Semen was diluted 1:40 with 2.9% sodium citrate dihydrate and the optical density of the diluted samples was read in a Bausch and Lomb Spectronic 20 Spectrophotometer with the wavelength set at 530 mu. The optical density reading was converted to sperm concentration using a regression equation. The regression equation used to estimate sperm concentration (Y) from optical density readings (X) was Y = 740.8 x -38.3 (Shelby, 1966).

Semen pH was determined by a portable Corning, battery-operated Model 7 pH meter with a plastic-sheathed combination electrode.

Experimental Gilts and Management

Twenty-nine (trial I) and 42 (trial II) crossbred gilts of similar nutritional and managerial environment were bred artificially in this phase of the study. All gilts were fed approximately 2.5 kg per head daily of the same ration as the boars (table 1).

The average age of all gilts at time of insemination was 283.2 ± 16.8 days and 238.7 ± 10.0 days for trials I and II,

respectively. Gilts were maintained in groups of 12 to 16 in a drylot of approximately 21.0 x 51.2 meters during the experimental period. A 2.44 x 3.66 meter shelter was available to the gilts in each pen. Gilts were checked twice daily, morning and evening, for symptoms of estrus with a mature teaser boar. Symptoms of estrus included mounting other gilts, perked ear response, rigidity to pressure on the loin, pursuit of the boar and willingness to stand. Gilts were considered ready to breed when they would stand for the boar or exhibited rigid immobility for the "back pressure test."

Since fresh semen was to be used and it is recommended that whole boar semen be utilized within 2 hours after collection, it was not possible to breed all gilts 12 hours after they were observed in standing estrus due to (1) only being able to collect boars once per day, (2) low volume of ejaculates collected and (3) the number of gilts exhibiting estrus the same day. Therefore, gilts were randomly bred within treatment at 12 or 24 hours after being observed in standing estrus.

Gilts were inseminated once with 50 ml of whole homospermic or heterospermic semen with a 50 ml disposable syringe connected to a plastic insemination rod (5 mm x 40.6 cm) with rubber tubing. The semen was deposited during a time period of approximately 5 minutes. Gilts were assigned randomly as they came into estrus to their respective treatment groups.

Processing Semen for Insemination

Succeeding a semen evaluation, the ejaculates were used in preparing either a homospermic or heterospermic insemination sample. The homospermic sample was composed of 50 ml of whole semen from each of the boars. There was no attempt to have an equal number of spermatozoa supplied by each boar in the heterospermic sample. Semen samples were placed in 60 ml polyethylene, Nalgene bottles with screw caps for transport to the insemination area.

Data Collected From Gilts

Gilts were slaughtered at a mean gestation length of 33.7 (trial I) and 42.2 (trial II) days and the reproductive tracts examined for number of embryos, corpora lutea and follicles. Macroscopically visible surface follicles were subjectively estimated for diameter and then punctured to prevent recounting. For the purpose of statistical analysis, the follicle diameter frequencies were grouped arbitrarily as follows: small follicles (2 to 4 mm) and medium follicles (5 to 10 mm).

The duration of the experimental periods were from June 6, 1974, to July 31, 1974 (trial I) and from October 23, 1974, to December 18, 1974 (trial II).

Experiment 2

The objectives of this experiment were to accustom boars to collection procedures and to obtain data on their general semen quality during development of the techniques and procedures to be used in experiment 3.

One Yorkshire and one Hampshire boar were used in this study. The age of the boars was approximately 24 months and 17 months for the Yorkshire and Hampshire, respectively.

During the months from September to December, 1975, the sperm-rich and post-sperm fractions of the ejaculates were collected at a minimum of 48-hour intervals. The gelatinous material was removed in all collections by filtration through sterile gauze. Each ejaculate was characterized for pH and volume of post-sperm, volume of sperm-rich and total volume collected. The sperm-rich fraction was further characterized for pH, concentration, primary and secondary abnormalities and motility. Primary abnormalities occurring in spermatozoa are due to metamorphic defects happening in the seminiferous tubules during the formation of spermatozoa from spermatocytes. Defects classified as primary abnormalities were giant heads, double heads, slender heads and double middle piece and tail. Abnormalities arising from environmental conditions after spermatozoa depart from the seminiferous tubules are known as secondary abnormalities. Abnormalities of this type are loose heads, cytoplasmic droplets and coiled and bent tails.

Experiment 3

The objectives of this experiment were to study the effects of mixing various semen components involving spermatozoa in the sperm-rich and seminal plasma from the post-sperm fractions from two boars and Beltsville Ll extender on oxygen consumption, pH, motility and percent dead spermatozoa. The effect of storage on oxygen consumption, pH,

motility and percent dead spermatozoa in the various combinations was investigated. Table 2 outlines the experimental design used in this investigation. The composition of BLl extender developed by Pursel et al. (1973, 1974) for boar semen is shown in table 3.

Management of Boars

The boars were housed and fed in the same manner as the boars in experiment 1.

Semen from two mature boars (one Hampshire and one Yorkshire) was collected by the gloved-hand technique (Herrick and Self, 1962) and fractionated visually into sperm-rich and post-sperm portions. Both the sperm-rich and post-sperm fractions were used in this experiment. Collection vessels used were four 1 pint Thermos flasks (Model 7207) with removable liners. All thermoses were maintained in an incubator set at 42 C when not being used. At the time of preparing the thermos for collection, the plastic inner liner was removed and 38 C water was poured into the glass insulated shell followed by replacement of the liner. Prior to collecting the ejaculate, a double layer of cheese cloth was placed over the opening to remove the gelatinous portion of the ejaculate. Immediately following collection of the last boar, semen was transported to the laboratory and placed in a water bath at 38 C until further processing.

A minimum interval of 48 hours was allowed between collections from each boar.

TABLE 2. EXPERIMENTAL DESIGN FOR EXPERIMENT 3

Plasma	Fresh spermatozoa			Stored spermatozoa		
	HSR	YSR	HSR + YSR	HSR	YSR	HSR + YSR
HPSP	1[a]	2	3	1	2	3
YPSP	4	5	6	4	5	6
HPSP + YPSP	7	8	9	7	8	9
BL1	10	11	12	10	11	12
HPSP + BL1	13	14	15	13	14	15
YPSP + BL1	16	17	18	16	17	18
HPSP + YPSP + BL1	19	20	21	19	20	21

[a] Treatment number.

TABLE 3. COMPOSITION OF BELTSVILLE L1 EXTENDER

Ingredient[a]	Quantity
Glucose	2.90 g
Sodium citrate dihydrate	1.00 g
Sodium bicarbonate	.20 g
Potassium chloride	.03 g
Dihydrostreptomycin sulfate	.10 g
Potassium penicillin G	1000 IU/ml

[a] Ingredients dissolved and brought to 100 ml with distilled water.

Semen Evaluation

Sperm motility was subjectively estimated by placing a small drop of semen on a plain microscope slide with a Pasteur disposable pipette and observed under low power magnification (100X) of a light microscope. Percent progressive motility was estimated on a scale of 0 to 100% in 5% intervals.

Sperm morphology and live-dead classifications were made under high power magnification (400X) of a Nikon Phase-Contrast microscope. The live-dead stain described in appendix A was used in this experiment for both live-dead and morphology classifications. A small drop of semen and stain was placed 1 cm from one end on a microscope slide with Pasteur disposable pipettes and mixed thoroughly. After mixing the slide used to draw out the film was lowered at a 45 degree angle to the horizontal slide and moved forward until the sample was spread evenly across the end of the angled slide. Then the angle slide was pulled backward until the semen sample spread out on the horizontal slide. Ten cells in 10 different fields were counted and expressed as a percentage of the total.

Sperm concentration of the sperm-rich fraction was determined by microscopically counting spermatozoa on a Neubauer hemacytometer. The pH of all semen samples was determined by a Beckman Model Zeromatic II pH meter using a micro combination electrode.

The volume of sperm-rich and post-sperm was measured in a 100 ml graduated cylinder.

Preparation of Treatments

Upon completion of a semen evaluation, the ejaculates were used to prepare treatment combinations. The post-sperm fraction of each boar was placed in a 250 ml polypropylene centrifuge bottle with screw cap and centrifuged at 300g for 10 minutes to eliminate spermatozoa in this fraction. The seminal plasma was removed from the compacted spermatozoa by decantation and the volume recorded for use in later calculations.

A total volume of 12 ml of sample per treatment with a final concentration of 1×10^8 motile spermatozoa per ml was desired. Therefore, equation 1 was utilized in determining the number of ml to use from the Hampshire or Yorkshire sperm-rich in preparing treatments with only one boar's spermatozoa.

Equation 1:

$$\text{Conc. } (10^8/\text{ml}) \times \text{Motility} \times (X) = 12 \text{ ml}$$

The amount of diluent (seminal plasma or BL1) to be added to the calculated amount in equation 1 to have a concentration of 1×10^8 per ml when dealing with only one boar's spermatozoa was accomplished by equation 2.

Equation 2:

$$\frac{\text{Conc. } (10^8/\text{ml}) \times \text{Motility} \times \text{Vol. SR}}{1 \times 10^8} - \text{Vol. SR} = \text{Vol. Diluent to add to SR}$$

When a treatment was to have both boars' spermatozoa represented, the calculated ml of sperm-rich in equation 1 was divided by two for each boar. This results in the same number of total motile spermatozoa being supplied by each boar.

The calculated ml of diluent in equation 2 was divided by two or three when a treatment required two or three different diluents, respectively. Consequently, this provides the same volume of diluent from each source.

Reference is given to appendix B for a more detailed explanation of treatment preparation.

After treatment samples were prepared, 2 ml of fresh sample were used for oxygen consumption and 6 ml were used for the storage sample. Prior to taking the 6 ml for storage, an initial estimate of motility, initial pH measured and an initial live-dead stain were prepared on each treatment.

Employment of Manometry

All semen samples were incubated in Warburg flasks in a Gilson constant volume respirometer under aerobic conditions. Volume of the Warburg vessels plus manometers was determined by the method of Lazarow (1951) using a micrometer calibrator. Oxygen consumption of semen samples (10^8 sperm per ml) was measured in microliters per 10^8 spermatozoa by the direct method of Warburg as described by Umbreit et al. (1972). The method of calculating the microliters of oxygen uptake from the observed changes in the level of the manometer fluid of the thermobarometer and of the reaction flask manometer was performed by the interval uptake method. A computer program was developed to calculate the microliters of oxygen taken up during each interval and to accumulate over intervals to obtain a total sum. These computed

values were then punched onto a new deck of cards by the IBM computer and used for further computations and analysis.

Preliminary study showed that no significant differences existed between the two respirometers used in this experiment when oxygen uptake was measured with both machines set at the same temperature and shaking rate. The Warburg respirometers were set at 38 C and to shake the flasks 100 oscillations per minute moving 3.5 cm per stroke. The ground glass joints between the flasks and manometers were greased with anhydrous lanolin, and the flasks were attached to the manometers with steel springs. The water level in the incubation tank of the respirometer was maintained so that the flask and its ground glass joint were completely submerged.

Each treatment was assigned at random to one of 21 Warburg flasks which resulted in the treatments being randomized between machines. Two ml of semen from each treatment were placed in the main vessel of the respective flask with a 1 ml Biopette (Schwarz/Mann) and 500 ul of diethanolamine (DEA) mixture was placed in the side arm with a 500 ul Eppendorf pipette (Brinkman Instruments, Inc.). DEA is a substance developed by Pardee (1949) which binds acidic gases reversibly to maintain a low partial pressure of carbon dioxide in the atmosphere, thus allowing the measurement of oxygen uptake by the direct method of Warburg in the presence of low levels of carbon dioxide. Shelby (1966) and Sanford and King (1972) investigated the use of DEA for boar semen metabolism studies and found it to be adequate for use in metabolism work. The composition of the DEA

mixture developed by Pardee (1949) and described by Umbreit et al. (1972) is presented in table 4.

TABLE 4. COMPOSITION OF DIETHANOLAMINE MIXTURE

Ingredient	Quantity
Diethanolamine	120 ml
6N hydrochloric acid	44 ml
Potassium bicarbonate	60 g
Thiourea	300 mg
Distilled water	136 ml

The mixture used in this experiment provided a 1% carbon dioxide atmosphere within a Warburg flask. In equation 3 it can be seen that carbon dioxide formed by metabolism is removed by the reaction proceeding from left to right and carbon dioxide used is replaced by the reverse reaction.

Equation 3:

$$NH(CH_2CH_2OH)_2 + CO_2 + H_2O \rightleftharpoons HCO_3^- + {}^{+}NH_2(CH_2CH_2OH)_2$$

Since this type of reaction is taking place, it helps to prevent bicarbonate from being removed from the seminal plasma.

All 21 flasks with samples and two thermobarometer flasks were allowed to equilibrate in the water bath for 10 minutes before the stopcocks were closed for zero hour oxygen reading to be taken. Usually 96.4 ± 15 minutes had elapsed from the beginning of collection of the first boar to the closing of the stopcock on the first manometer. Readings were taken at 10-minute intervals for 12 times (µl I_1 to

µl I_{12}) and were accumulated for a total of 2 hours (µl A_2 to µl A_{12}). After the 2-hour incubation period, final motility estimates were made, final pH measured and a final live-dead stain prepared on all treatments.

Storage of Semen Samples

Six ml screw cap vials were used to store 6 ml of sample from the fresh sample preparations. All fresh semen samples were allowed to equilibrate with room temperature before being placed in a polystyrene container. The polystyrene container was then placed in a Forma Scientific Company Model 12 refrigerated incubator set at 15 ± 1 C. The duration of the in vitro storage was approximately 54 hours.

After the storage period the vials were removed from the refrigerated incubator and inverted 10 times. Two ml of semen sample were then placed in the same flask as the fresh semen sample was run. The flasks were connected to the manometers and submerged in the water bath immediately after the sample was placed in the flask. The Warburg was operated and read in the same manner as the fresh sample (µl I_1 to µl I_{12}, µl A_2 to µl A_{12}). The initial pH of the stored samples was determined after all flasks had been submerged in the water bath. Sample vials were than warmed for 30 minutes in a 38 C water bath before estimating initial motility and preparing a live-dead stain on the stored sample.

After 2 hours of incubation, final motility was estimated, final pH measured and a final live-dead stain prepared.

Statistical Analysis of Data

Statistical procedures in all experiments were performed according to Steel and Torrie (1960) and Snedecor and Cochran (1967). In this manuscript the maximum level of probability considered significant was 5%.

Experiment 1

Semen characteristics of the boars used in trials I and II were analyzed for significant differences utilizing a one-way analysis of variance. Least squares analysis of variance of the gilt data was performed where applicable. When there were significant differences among treatments as indicated by the F-test, Duncan's multiple range test was used for mean comparisons. Pregnancy rate was considered as one dichotomous variable and analyzed by chi-square.

Experiment 2

Semen characteristics between boars were analyzed by one-way analysis of variance. An F-test was used to detect significant differences.

Experiment 3

Total microliters of oxygen consumption, pH, motility and percent dead spermatozoa were analyzed by the least squares analysis of variance procedures. A set of orthogonal comparisons was made when the analysis of variance revealed a significant difference between treatments.

Polynominal regressions were performed to determine linearity of accumulated microliters of oxygen consumption and microliters of

oxygen taken up per interval for both fresh and stored semen samples. An analysis of variance was employed to determine if there was a difference between the fresh and stored regression lines.

Correlation coefficients for pH, motility and percent dead spermatozoa were obtained by linear regression methods.

Semen characteristic differences were analyzed by one-way analysis of variance.

RESULTS AND DISCUSSION

Experiment 1

Boar Data

Mean values of semen characteristics of the two boars utilized in trials I and II are shown in table 5.

Trial I. Semen characteristics analyzed were total volume, sperm concentration and pH of total ejaculate. Motility was observed periodically throughout trial I and found to be above 70% for both boars. Seventy percent motility is within the range (50 to 80%) reported by Foote (1974) for boar spermatozoa. Sperm concentration (10^6) was different ($P < .05$) between the Yorkshire and Hampshire (101.4 vs 147.7). Age and breed could be involved in this difference. Swierstra (1973) found the concentration (10^6) to be lower ($P < .01$) in boars 21 to 26 weeks of age than boars between 27 to 31 weeks of age (108.0 vs 163.8). The initial breed difference reported by Swierstra (1973) disappeared when boars reached 9 to 11 months of age.

Mean values for total volume of ejaculate and pH were not different between boars. Shelby (1966) and Singleton (1968, 1970) have reported higher values for semen pH in boars.

Trial II. The Yorkshire boar produced a larger ($P < .005$) total volume of ejaculate than the Hampshire boar (173.4 vs 127.9 ml). This difference is due to the larger ($P < .01$) post-sperm volume being secreted by the Yorkshire (128.0 vs 92.1 ml). McKenzie et al. (1938)

TABLE 5. MEANS AND STANDARD DEVIATIONS OF SEMEN CHARACTERISTICS OF BOARS - TRIALS I AND II

Characteristics	Boars[a] Hampshire (mean)	Yorkshire (mean)
	Trial I	
Total volume (ml)	111.6 ± 10.9	122.4 ± 19.9
pH of semen	7.48 ± .14	7.49 ± .18
Conc. (10^6/ml)	147.7 ± 48.3[b]	101.4 ± 53.5[c]
	Trial II	
Total volume (ml)	127.9 ± 19.3[d]	173.4 ± 33.5[e]
Volume SR (ml)	35.9 ± 12.4	45.4 ± 17.6
Volume PS (ml)	92.1 ± 16.4[f]	128.0 ± 32.7[g]
Conc. SR (10^7/ml)	26.65 ± 8.2	27.66 ± 11.6
Conc. total ejaculate (10^7/ml)	8.59 ± 6.73	9.12 ± 3.84
Motility (%)	73.5 ± .05	74.3 ± .14
pH SR	7.20 ± .15	7.21 ± .20
pH PS	7.24 ± .12	7.17 ± .23

[a] The means are calculated from 14 and 15 collections per boar in trials I and II, respectively.
[b,c] Means within rows are significantly different, $P < .05$.
[d,e] Means within rows are significantly different, $P < .005$.
[f,g] Means within rows are significantly different, $P < .01$.

stated that accessory sex glands produce approximately 98% of the ejaculate volume. Therefore, post-sperm volume is, to a large extent, a measure of accessory sex gland function and responsible for differences in ejaculate volume between boars. Volume and concentration of the sperm-rich fraction did not differ between the two boars. A difference ($P < .05$) in sperm concentration in trial I and not in trial II supports the findings of Swierstra (1973) that boars do not differ in sperm concentration after 9 to 11 months of age. Boar age in trial I was 14 months for Hampshire and 8 months for the Yorkshire. In trial II the boars were 4 months older.

Motility estimates and pH measurements were found to be non-significant between the two boars. Lower pH values were recorded in trial II than in trial I. Post-sperm pH values are similar to the pH value (7.2) reported by Mann (1954) for seminal vesicle fluid secretions. Motility estimates were lower than estimations by Shelby and Foley (1966) but higher than estimates made by Swierstra (1973).

In general, the values obtained in trials I and II for semen characteristics appear to be acceptable and similar to those reported by other investigators.

Gilt Data

Mean squares for corpora lutea, follicles 2 to 4 mm, follicles 5 to 9 mm, total follicles, embryos and embryo to corpora lutea ratio are presented in table 6 for 30 pregnant gilts. The difference in total

TABLE 6. MEAN SQUARES FOR CORPORA LUTEA (CL), FOLLICLES, EMBRYOS AND EMBRYO TO CL RATIO IN PREGNANT GILTS

Source	df	Corpora lutea	Follicles			Embryo	Embryo:CL ratio
			2-4 mm	5-9 mm	Total		
Treatment (T)	2	5.033	22.845	121.366*	266.821*	46.716*	.158
Trial (t)	1	2.593	334.960	116.026*	955.164**	25.249	.140
T x t	2	14.515	112.845	36.554	31.452	9.379	.049
Error	24	4.856	91.847	22.043	78.148	9.670	.052

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

follicles ($P < .01$) and follicles 5 to 9 mm ($P < .05$) between trials is due to age of gilts. The average age of gilts in trial I was 283.2 days and in trial II 238.7 days. Gilts used in trial II were younger and appeared to have ovaries producing less follicles 5 to 9 mm (2.5 vs 6.8) and total follicles (26.6 vs 38.9) than older gilts. Least squares means for follicles 2 to 4 mm, follicles 5 to 9 mm, total follicles, corpora lutea, embryo to corpora lutea ratio and embryos are shown in table 7.

Duncan's multiple range test was made for those traits shown to be significantly different between groups of gilts. The number of follicles and corpora lutea is not a result of treatment. However, they were analyzed to determine differences between treatment groups of gilts. Gilts inseminated with straight Yorkshire semen had more ($P < .05$) total follicles (39.0) than gilts inseminated with Hampshire semen (28.6) or mixed semen (30.6). Although gilts inseminated with Yorkshire semen had more active ovaries, there was no difference in the number of corpora lutea between the groups of gilts. The number of corpora lutea recorded for the three groups of gilts is similar to the number reported by other investigators for corpora lutea in gilts (Edwards et al., 1968; Bayer et al., 1972; Edey et al., 1972). The number of recorded embryos was greater in the MS group of gilts than in the YS ($P < .01$) or HS ($P < .05$) group of gilts. The average between the HS and YS groups was 3.75 embryos less than the MS group. In addition, a higher ($P > .05$) embryo to corpora lutea ratio was found for the MS group.

TABLE 7. LEAST SQUARES MEANS AND STANDARD ERRORS FOR SELECTED CHARACTERISTICS IN PREGNANT GILTS (TRIALS I AND II)

Characteristics	Treatment groups 1 Yorkshire semen	2 Hampshire semen	3 Mixed semen
Number of gilts	10	8	12
Follicles, 2-4 mm	29.2 ± 3.0	25.8 ± 3.9	28.3 ± 2.9
Follicles, 5-9 mm	8.7 ± 1.5[a]	2.9 ± 1.9[b]	2.3 ± 1.4[b]
Total follicles	39.0 ± 2.8[c]	28.6 ± 3.6[d]	30.6 ± 2.7[d]
Corpora lutea (CL)	12.7 ± .7	12.8 ± .9	14.0 ± .7
Embryo to CL ratio	.45 ± .07	.51 ± .09	.70 ± .07
Number of embryos[e]	5.7 ± .1	6.4 ± 1.3	9.8 ± .9

[a,b] Means within the same row with different superscripts are different ($P < .01$).

[c,d] Means within the same row with different superscripts are different ($P < .05$).

[e] Treatment 3 greater than treatment 1 ($P < .01$) and treatment 2 ($P < .05$). No difference between treatments 1 and 2.

Assuming no more embryonic and/or fetal losses in this study, the results of this study do not agree with other published data (Hlebov, 1965; Cerne and Salehar, 1964) that heterospermic semen produces a lower litter size. A lower number of embryos was recorded for the YS and HS groups of gilts than average litter size for gilts (8.7 to 10.9 embryos) reported in the literature (Pursel et al., 1973; Edwards et al., 1968). Embryonic mortality (based on embryo:CL ratio) tended to be less in gilts inseminated with heterospermic semen (30%) than gilts bred with HS (49%) or YS (55%), assuming no difference in

fertilization rate. Gilts bred with YS or HS had a higher embryonic mortality rate than normally (33%) found (Anderson, 1974).

Chi-square analysis indicated no difference in pregnancy rate between YS (43%), HS (33%) or MS (50%) groups. Cerne and Salehar (1964) reported no difference in pregnancy rate between heterospermic and homospermic semen. However, Hlebov (1965), Vasiljev et al. (1968) and Popenko (1969) have shown higher pregnancy rates for heterospermic semen.

Pregnancy rates for gilts have been shown in the literature to be higher (Koch et al., 1976; Pursel et al., 1973) and lower (Borton et al., 1965; Self, 1961) than found in this study. Inseminating gilts only once may be part of the reason for not obtaining a higher pregnancy rate. Borton et al. (1965) indicated a higher pregnancy rate when gilts were inseminated twice in one estrous cycle. The influence of semen backflow following insemination was not considered to be a cause of low pregnancy rate. Borton et al. (1965) found a peculiar phenomenon that pregnancy rate was higher ($P < .01$) when some backflow occurred after inseminating gilts with 50 ml of semen. In contrast, other investigators reported no relationship between the volume of semen lost during insemination and pregnancy rate or litter size (Dziuk, 1958; Madden, 1959; Radford, 1961).

The volume (50 ml) of semen used in this study could possibly have affected pregnancy rate. Volumes larger than 50 ml have been reported to give better pregnancy rates (Rotke, 1960; Polge, 1959; First et al., 1960; Du Mesnil du Buisson and Signoret, 1970), although

other investigators have found no advantage of larger volumes on pregnancy rate (Wiggens et al., 1951; Self, 1959; Stratman, 1961; Radford, 1965).

Since the gilts were only inseminated once with 50 ml of semen, a more critical evaluation of mixed semen versus homospermic semen has been made. It appears that heterospermic semen did increase the number of viable embryos but did not have an effect on pregnancy rate.

Experiment 2

These boars are not the same two used in experiment 1. Means and standard deviations for semen characteristics are shown in table 8. Characteristics measured were all significantly different between the two boars, except for primary and secondary abnormalities and percent dead spermatozoa. The Hampshire boar produced a larger ($P < .05$) volume of sperm-rich (100.0 vs 85.6 ml), post-sperm (232.9 vs 184.8 ml) and total volume (333.0 vs 270.4 ml) when compared to the Yorkshire boar. Both boars produced a larger volume of sperm-rich than reported by Singleton (1970). The average total volumes produced by the boars are within the range of values (176 to 323 ml) reported by other investigators (Pickett et al., 1967; Swierstra and Rahnefeld, 1967; Swierstra, 1970, 1974; Swierstra and Dyck, 1976).

The volume of centrifuged post-sperm plasma indicated approximately 3.4% and 7.8% of the post-sperm fraction was spermatozoa for the Hampshire and Yorkshire, respectively. Motility estimates of Yorkshire semen (82%) was better ($P < .05$) than Hampshire semen (73.3%). Motility

TABLE 8. MEANS AND STANDARD DEVIATIONS FOR SEMEN CHARACTERISTICS OF TWO BOARS COLLECTED 15 TIMES

Characteristic	Boar	
	Hampshire (mean)	Yorkshire (mean)
Volume SR (ml)	100.0 ± 21.0[a]	85.6 ± 16.9[b]
Volume PS (ml)	232.9 ± 61.0[a]	184.8 ± 67.4[b]
Total volume (ml)	333.0 ± 74.7[a]	270.4 ± 76.8[b]
Volume CPS (ml)	224.9 ± 60.3[a]	170.4 ± 61.0[b]
Motility (%)	75.3 ± .06[a]	82.0 ± .1[b]
Conc. SR (10^7/ml)	35.4 ± 14.7[c]	60.2 ± 22.3[d]
pH SR	7.39 ± .17[c]	7.61 ± .16[d]
pH PSP	7.37 ± .15[c]	7.71 ± .12[d]
Total primary abnormalities (%)	1.25 ± .55	1.50 ± .80
Total secondary abnormalities (%)	6.25 ± 3.08	4.42 ± 2.31
Dead sperm	14.08 ± 4.25	16.42 ± 6.75

[a,b] Means within rows are significantly different, $P < .05$.
[c,d] Means within rows are significantly different, $P < .005$.

estimates have been documented to be different between boars (Singleton, 1968).

Sperm concentration and pH measurements were found to be different ($P < .005$) between the two boars. Spermatozoa concentration of the whole ejaculate in boars has been reported to be approximately 150 to 250 x 10^6 and 510 to 600 x 10^6 in the sperm-rich fraction (Borton et al., 1965; Radford, 1965). Therefore, the mean concentration of the Hampshire sperm-rich (354 x 10^6) is lower than published values, while the Yorkshire has a higher sperm concentration (602 x 10^6) than published values. The pH values of Yorkshire semen are similar to those reported by Foley et al. (1964), Shelby (1966) and Singleton (1970). Although lower than Yorkshire semen, Hampshire semen pH values are within the range of pH values (7.3 to 7.8) reported by White (1974).

Since semen characteristics did differ between the two boars, they were considered to be adequate for use in experiment 3. The objectives of experiment 3 were to study the effects of mixing various semen components of two boars on oxygen consumption, pH, motility and percent dead spermatozoa. Boars with different semen characteristics should give a better evaluation of heterospermic effects than boars of identical characteristics.

Experiment 3

Semen Characteristics

Means for semen characteristics of the boars collected eight times for experiment 3 are shown in table 9. Although the values are different between tables 8 and 9, the same general trend is observed.

TABLE 9. MEANS AND STANDARD DEVIATIONS FOR SEMEN CHARACTERISTICS OF BOARS COLLECTED EIGHT TIMES EACH FOR EXPERIMENT 3

Characteristic	Boar	
	Hampshire (mean)	Yorkshire (mean)
Volume SR (ml)	77.8 ± 15.7[a]	67.1 ± 8.2[b]
Volume PS (ml)	229.4 ± 84.0[a]	101.0 ± 27.5[b]
Total volume (ml)	307.1 ± 85.9[a]	168.1 ± 26.9[b]
Motility (%)	83.1 ± 4.6[c]	89.4 ± 1.8[d]
pH SR	7.36 ± .08[a]	7.94 ± .10[b]
pH PS	7.28 ± .08[a]	7.98 ± .18[b]
pH CPS	7.39 ± .07[a]	8.15 ± .10[b]
Total primary abnormalities (%)	1.13 ± .35	1.50 ± .76
Total secondary abnormalities (%)	7.13 ± 2.23	5.38 ± 2.20
Dead sperm (%)	18.5 ± 3.9	16.9 ± 4.8
Conc. SR (10^7/ml)	36.6 ± 14.1[a]	107.6 ± 47.9[b]
Total motile sperm in SR (10^9/ml)	23.1 ± 8.7	45.6 ± 24.6

[a,b] Means within rows are significantly different, $P < .005$.
[c,d] Means within rows are significantly different, $P < .01$.

The Hampshire produced a larger ($P < .005$) volume of sperm-rich, post-sperm and total volume when compared to the Yorkshire. Yorkshire semen had higher pH values ($P < .005$) and motility estimates ($P < .01$). After centrifugation the pH values of the post-sperm fraction increased in both boars. The increase in pH is probably due to a loss of bicarbonate. If the bicarbonate in the semen cannot equilibrate with the carbon dioxide in the surrounding atmosphere, the pH of the semen becomes progressively more alkaline due to the loss of bicarbonate (Shelby, 1966). Total primary and secondary abnormalities and percent dead spermatozoa were not different between boars.

Spermatozoa concentration in the sperm-rich fraction was different ($P < .005$) between the Hampshire (36.6×10^7) and Yorkshire (107.6×10^7). The average sperm concentration of the Yorkshire is exceptionally high, when considering published values of 510 to 600 x 10^6 for the sperm-rich fraction (Borton et al., 1965). Spermatozoa concentration (10^7) for the Hampshire ranged from 20 to 60. Total motile spermatozoa in the sperm-rich fraction was not different between the Yorkshire and Hampshire (45.6×10^9 vs 23.1×10^9).

Warburg Results

Mean squares for total microliters of oxygen consumption are presented in table 10. Differences ($P < .005$) did exist for treatments (T), fresh vs stored (F vs S) and days (D). However, these differences may be of limited value due to interactions of T x F vs S ($P < .005$), T x D ($P < .005$) and F vs S x D ($P < .05$). The biological explanation for these interactions is not known.

TABLE 10. MEAN SQUARES FOR OXYGEN CONSUMPTION (TOTAL MICROLITERS)

Source	df	Mean squares
Treatment (T)	20	4332.163***
Fresh vs stored (F vs S)	1	10947.28 ***
Days (D)	7	8437.29 ***
T x F vs S	20	1589.26 ***
T x D	140	599.16 ***
F vs S x D	7	669.92 *
F vs S x T x D	140	288.81

* $P < .05$.
*** $P < .005$.

A quadratic ($P < .05$) polynomial regression for accumulative microliters of oxygen consumed was found for fresh semen samples, whereas a linear relationship was observed for accumulative microliters of oxygen consumed in stored samples. The regression lines (linear) between fresh and stored semen were different ($P < .01$). Least squares means for total microliters of oxygen consumed over all treatments were 63.3 ml for fresh semen and 51.8 ml for stored semen. A regression was performed on microliters of oxygen taken up per interval and found to be cubic ($P < .05$) in stored semen samples and linear in fresh semen samples. Regression lines (linear) for the fresh and stored semen samples were different ($P < .05$). Statistical analysis was not performed on the data for µl of oxygen taken up per interval due to high cost.

It is evident from table 11 that the quantity of oxygen consumed by the spermatozoa within the treatments varies between days. This difference could be expected since boar semen characteristics have been shown to vary between boars (Swierstra, 1973). Singleton (1968) observed differences ($P < .01$) between boars and between ejaculates within boars for oxygen consumption and semen characteristics.

TABLE 11. LEAST SQUARES MEANS FOR TOTAL MICROLITERS OF OXYGEN CONSUMED OVER ALL TREATMENTS (DAY EFFECT)

Day of collection	Means (µl)[a]		
	Fresh	Stored	Combined
1	43.04	37.74	40.39^{bc}
2	66.14	53.72	59.93^{b}
3	60.61	33.71	47.16^{bc}
4	63.04	54.83	58.93^{b}
5	61.08	61.38	61.23^{b}
6	50.07	37.64	43.86^{bc}
7	90.31	79.76	85.04^{d}
8	71.76	55.94	63.85^{b}

[a] Means for fresh and stored columns were not analyzed statistically due to interactions.

[b,c,d] Means within column with different superscripts differ significantly, $P < .01$.

Least squares means for total microliters of oxygen consumed for treatment by storage interactions ($P < .005$) are presented in table 12. Since 21 treatments are involved, the interaction is difficult to interpret. A portion of the interaction can be explained on the basis that some treatments decreased and others increased in oxygen consumption after storage. Treatments 1, 2, 3, 10, 13 and 15 had increases in oxygen consumption of 24.08, 17.96, 29.56, 3.56, 2.34 and 1.16 µl, respectively, after storage when compared to the fresh sample. Other treatments used from 2.68 to 36.46 µl less oxygen after storage as compared to fresh samples. Treatments composed of HSR + HPSP, YSR + YPSP and HSR + YSR + HPSP used more oxygen after storage than any other treatment composition before or after storage. Treatment 9 (HSP + YSR + HPSP + YPSP) had the greatest reduction in oxygen consumption after storage.

Tables 13 and 14 present single degree orthogonal comparisons for total oxygen consumption for fresh and stored semen samples, respectively. It should be kept in mind that these two tables may be of limited value due to significant interactions.

Fresh Sample. Within the spermatozoa comparisons no difference was found between straight HS and YS vs mixed spermatozoa for oxygen consumption. The comparison of HS vs YS was not significant. Hampshire spermatozoa reacted differently ($P < .01$) in the comparison of treatments 1, 4 and 7 vs 10, 13, 16 and 19. Diluents containing BL1 resulted in a lower amount of oxygen consumed (58.22 vs 73.89 µl). When YPSP was used as a dilution agent for HS, a reduction ($P < .05$) in

TABLE 12. LEAST SQUARES MEANS FOR OXYGEN CONSUMPTION FOR TREATMENT BY STORAGE INTERACTION (FRESH AND STORED SAMPLES)

Treatment composition	Treatment number	Means (µl/2 hours) Fresh	Means (µl/2 hours) Stored	Change in µl
HSR + HPSP	1	78.17	102.25	+24.08
HSR + YPSP	4	61.51	25.65	-35.86
HSR + HPSP + YPSP	7	81.99	51.87	-30.12
HSR + BL1	10	52.27	55.83	+ 3.56
HSR + HPSP + BL1	13	58.45	60.79	+ 2.34
HSR + YPSP + BL1	16	62.71	50.04	-12.67
HSR + HPSP + YPSP + BL1	19	59.44	56.76	- 2.68
YSR + YPSP	2	84.19	102.15	+17.96
YSR + HPSP	5	40.75	15.04	-25.71
YSR + HPSP + YPSP	8	68.43	36.05	-32.38
YSR + BL1	11	53.06	48.27	- 4.79
YSR + HPSP + BL1	14	67.22	61.49	- 5.73
YSR + YPSP + BL1	17	60.87	33.29	-27.58
YSR + HPSP + YPSP + BL1	20	69.18	37.03	-32.15
HSR + YSR + HPSP	3	72.08	101.64	+29.56
HSR + YSR + YPSP	6	51.96	18.76	-33.20
HSR + YSR + HPSP + YPSP	9	77.51	41.05	-36.46
HSR + YSR + BL1	12	54.81	45.04	- 9.77
HSR + YSR + HPSP + BL1	15	62.08	63.24	+ 1.16
HSR + YSR + YPSP + BL1	18	52.17	35.29	-16.88
HSR + YSR + YPSP + HPSP + BL1	21	59.52	47.11	-12.41

TABLE 13. LEAST SQUARES MEANS FOR TOTAL OXYGEN CONSUMPTION (FRESH SAMPLE)

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means (µl per 2 hours)
Total treatments		20		
	Spermatozoa Comparisons			
HS, YS vs HS+YS	1,4,7,10,13,16, 19,2,5,8,11,14, 17,20 vs 3,6,9, 12,15,18,21	1	ns	64.16 vs 61.45
HS vs YS	1,4,7,10,13,16, 19 vs 2,5,8,11, 14,17,20	1	ns	64.93 vs 63.39
	Plasma Comparisons for Hampshire Sperm			
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+BL1, HPSP+ YPSP+BL1	1,4,7 vs 10,13, 16,19	1	.01	73.89 vs 58.22
HPSP, YPSP vs HPSP+YPSP	1,4 vs 7	1	ns	69.84 vs 81.99
HPSP vs YPSP	1 vs 4	1	.05	78.17 vs 61.51
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	10 vs 13,16,19	1	ns	52.27 vs 60.20
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	13,16 vs 19	1	ns	60.58 vs 59.44
HPSP+BL1 vs YPSP+BL1	13 vs 16	1	ns	58.45 vs 62.71

TABLE 13 CONTINUED

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means (µl per 2 hours)
Plasma Comparisons for Yorkshire Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	2,5,8 vs 11,14, 17,20	1	ns	64.46 vs 62.58
HPSP, YPSP vs HPSP+YPSP	2,5 vs 8	1	ns	62.47 vs 68.43
HPSP vs YPSP	2 vs 5	1	.01	84.19 vs 40.75
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	11 vs 14,17,20	1	ns	53.06 vs 65.76
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	14,17 vs 20	1	ns	64.05 vs 69.18
HPSP+BL1 vs YPSP+BL1	14 vs 17	1	ns	67.22 vs 60.87
Plasma Comparisons for Mixed Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	3,6,9 vs 12,15, 18,21	1	.05	67.18 vs 57.15
HPSP, YPSP vs HPSP+YPSP	3,6 vs 9	1	.05	62.02 vs 77.51
HPSP vs YPSP	3 vs 6	1	.05	72.08 vs 51.96
BL1 vs HPSP+BL1, YPSP+ HPSP+YPSP+BL1	12 vs 15,18,21	1	ns	54.81 vs 57.92
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	15,18 vs 21	1	ns	57.13 vs 59.52
HPSP+BL1 vs YPSP+BL1	15 vs 18	1	ns	62.08 vs 52.17

TABLE 14. LEAST SQUARES MEANS FOR TOTAL OXYGEN CONSUMPTION (STORED SAMPLE)

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means (µl per 2 hours)
Total treatments		20		
	Spermatozoa Comparisons			
HS, YS vs HS+YS	1,4,7,10,13,16, 19,2,5,8,11,14, 17,20 vs 3,6,9, 12,15,18,21	1	ns	52.61 vs 50.30
HS vs YS	1,4,7,10,13,16, 19 vs 2,5,8,11, 14,17,20	1	.005	57.60 vs 47.62
	Plasma Comparisons for Hampshire Sperm			
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	1,4,7 vs 10,13, 16,19	1	ns	59.92 vs 55.76
HPSP, YPSP vs HPSP+YPSP	1,4 vs 7	1	ns	63.95 vs 51.87
HPSP vs YPSP	1 vs 4	1	.005	102.25 vs 25.65
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	10 vs 13,16,19	1	ns	55.83 vs 55.86
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	13,16 vs 19	1	ns	55.42 vs 56.76
HPSP+BL1 vs YPSP+BL1	13 vs 16	1	ns	60.79 vs 50.04

TABLE 14 CONTINUED

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means (µl per 2 hours)
Plasma Comparisons for Yorkshire Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	2,5,8 vs 11,14, 17,20	1	ns	51.08 vs 45.02
HPSP, YPSP vs HPSP+YPSP	2,5 vs 8	1	.005	58.60 vs 36.05
HPSP vs YPSP	2 vs 5	1	.005	102.15 vs 15.04
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	11 vs 14,17,20	1	ns	48.27 vs 43.94
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	14,17 vs 20	1	ns	47.39 vs 37.03
HPSP+BL1 vs YPSP+BL1	14 vs 17	1	.005	61.49 vs 33.29
Plasma Comparisons for Mixed Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	3,6,9 vs 12,15, 18,21	1	ns	53.82 vs 47.67
HPSP, YPSP vs HPSP+YPSP	3,6 vs 9	1	.05	60.20 vs 41.05
HPSP vs YPSP	3 vs 6	1	.005	101.64 vs 18.76
BL1 vs HPSP+BL1, YPSP+ HPSP+YPSP+BL1	12 vs 15,18,21	1	ns	45.04 vs 48.55
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	15,18 vs 21	1	ns	49.29 vs 47.11
HPSP+BL1 vs YPSP+BL1	15 vs 18	1	.005	63.24 vs 35.29

oxygen consumption occurred as compared to the diluent HPSP. The YPSP also caused a decrease ($P < .01$) in oxygen consumption by YS. Other comparisons of plasma effects on HS or YS were not significant. Plasma effects on mixed spermatozoa were different. The comparisons of treatments 3, 6 and 9 vs 12, 15, 18 and 21 showed mixed spermatozoa using less ($P < .05$) oxygen when BL1 was present. Oxygen consumption was higher ($P < .05$) when diluting mixed spermatozoa with HPSP instead of YPSP (72.08 vs 51.96 µl).

Stored Sample. After storage there was no difference for the comparison HS, YS vs HS + YS. However, a difference ($P < .005$) did exist between the HS vs YS comparison (57.60 vs 47.62 µl). Hampshire spermatozoa diluted with YPSP used less ($P < .005$) oxygen than HS diluted with HPSP. There were no other differences found for plasma effects on HS after storage. When HPSP or HPSP + BL1 were used as a diluent for YS, more ($P < .005$) oxygen was consumed than when YPSP or YPSP + BL1 were used as a diluent. Diluting MS with HPSP or HPSP + BL1 resulted in more ($P < .005$) oxygen being consumed than when diluting with YPSP or YPSP + BL1.

Mean squares for pH, motility and percent live-dead spermatozoa before and after placement of semen samples on the Warburg apparatus are shown in table 15. All characteristics measured in both fresh and stored samples showed treatment differences, except for percent dead spermatozoa after removal from the Warburg in the fresh sample.

TABLE 15. MEAN SQUARES FOR pH, MOTILITY AND LIVE-DEAD SPERMATOZOA BEFORE AND AFTER INCUBATION ON WARBURG (FRESH AND STORED SAMPLES)

Source	df	pH		Motility		Percent dead	
		Before	After	Before	After	Before	After
				Fresh Sample			
Treatment	20	.2302***	.1775***	.0412***	.18678***	.0048*	.0029
Days	7	.1125***	.0664	.06318***	.3587***	.0079*	.0207**
Remainder	140	.0079	.0331	.0082	.0390	.0029	.0033
				Stored Sample			
Treatment	20	1.0410***	.3070***	.3257***	.2684***	.0089***	.0118**
Days	7	.4318***	.4429***	.0478***	.1889***	.0113***	.0109
Remainder	140	.0478	.0751	.0164	.0297	.0020	.0057

* $P < .05$.
** $P < .01$.
*** $P < .005$.

In fresh semen a difference ($P < .005$) existed in pH between HS (8.04) and YS (8.17) before samples were placed on the Warburg (table 16). This difference ($P < .05$) still existed when the samples were removed from the Warburg after 2 hours of incubation (table 17). When utilizing HPSP or YPSP as a diluent for HS, YS or MS, a higher ($P < .005$) pH value resulted from the YPSP before and after operation of the Warburg. Diluting HS, YS or MS with HPSP + BL1 or YPSP + BL1, a higher ($P < .05$) pH value was recorded for the YPSP + BL1 (table 16). The initial difference ($P < .005$) in treatment comparison 11 vs 14, 17 and 20 (8.01 vs 8.22) before placement of samples on the Warburg had disappeared (8.46 vs 8.41) when the treatments were removed from the Warburg (tables 16 and 17).

Motility estimates for fresh semen were lower ($P < .05$) for HS, YS and MS diluted with YPSP as compared to HPSP (tables 18 and 19). A larger difference between the HPSP and YPSP occurred after 2 hours of incubation than prior to incubation. When comparing treatments 2 and 5 vs 8, the YS diluted with HPSP + YPSP had a higher ($P < .005$) estimate of motility (82.5 vs 66.9) than YS diluted with HPSP or YPSP (table 18). This difference was not found after 2 hours of incubation (table 19). The same results occurred when MS was diluted with HPSP, YPSP or HPSP + YPSP (tables 18 and 19). A difference ($P < .05$) did occur in comparison of treatments 3, 6 and 9 vs 12, 15, 18 and 21 after incubation that was not present prior to incubation. It appears that BL1 caused a decrease in motility estimates (table 19).

TABLE 16. LEAST SQUARES MEANS FOR SEMEN pH BEFORE PLACEMENT ON WARBURG (FRESH SAMPLE)

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Total treatments		20		
	Spermatozoa Comparisons			
HS, YS vs HS+YS	1,4,7,10,13,16, 19,2,5,8,11,14, 17,20 vs 3,6,9, 12,15,18,21	1	ns	8.10 vs 8.15
HS vs YS	1,4,7,10,13,16, 19 vs 2,5,8,11, 14,17,20	1	.005	8.04 vs 8.17
	Plasma Comparisons for Hampshire Sperm			
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	1,4,7 vs 10,13, 16,19	1	ns	8.02 vs 8.05
HPSP, YPSP vs HPSP+YPSP	1,4 vs 7	1	ns	8.03 vs 8.01
HPSP vs YPSP	1 vs 4	1	.005	7.84 vs 8.22
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	10 vs 13,16,19	1	ns	8.08 vs 8.04
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	13,16 vs 19	1	ns	8.04 vs 8.04
HPSP+BL1 vs YPSP+BL1	13 vs 16	1	.05	7.96 vs 8.12

TABLE 16 CONTINUED

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Plasma Comparisons for Yorkshire Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	2,5,8 vs 11,14, 17,20	1	ns	8.18 vs 8.17
HPSP, YPSP vs HPSP+YPSP	2,5 vs 8	1	ns	8.18 vs 8.18
HPSP vs YPSP	2 vs 5	1	.005	7.89 vs 8.46
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	11 vs 14,17,20	1	.005	8.01 vs 8.22
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	14,17 vs 20	1	ns	8.23 vs 8.19
HPSP+BL1 vs YPSP+BL1	14 vs 17	1	.005	8.09 vs 8.37
Plasma Comparisons for Mixed Semen				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	3,6,9 vs 12,15, 18,21	1	ns	8.13 vs 8.17
HPSP, YPSP vs HPSP+YPSP	3,6 vs 9	1	ns	8.13 vs 8.13
HPSP vs YPSP	3 vs 6	1	.005	7.88 vs 8.38
BL1 vs HPSP+BL1, YPSP+ HPSP+YPSP+BL1	12 vs 15,18,21	1	ns	8.20 vs 8.15
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	15,18 vs 21	1	ns	8.15 vs 8.16
HPSP+BL1 vs YPSP+BL1	15 vs 18	1	.005	8.03 vs 8.27

TABLE 17. LEAST SQUARES MEANS FOR SEMEN pH AFTER REMOVAL FROM WARBURG (FRESH SAMPLE)

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Total treatments		20		
	Spermatozoa Comparisons			
HS, YS vs HS+YS	1,4,7,10,13,16, 19,2,5,8,11,14, 17,20 vs 3,6,9, 12,15,18,21	1	ns	8.42 vs 8.43
HS vs YS	1,4,7,10,13,16, 19 vs 2,5,8,11, 14,17,20	1	.05	8.39 vs 8.44
	Plasma Comparisons for Hampshire Sperm			
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	1,4,7 vs 10,13, 16,19	1	ns	8.38 vs 8.40
HPSP, YPSP vs HPSP+YPSP	1,4 vs 7	1	ns	8.40 vs 8.33
HPSP vs YPSP	1 vs 4	1	.005	8.16 vs 8.64
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	10 vs 13,16,19	1	ns	8.37 vs 8.41
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	13,16 vs 19	1	ns	8.37 vs 8.48
HPSP+BL1 vs YPSP+BL1	13 vs 16	1	.05	8.28 vs 8.46

TABLE 17 CONTINUED

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Plasma Comparisons for Yorkshire Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	2,5,8 vs 11,14, 17,20	1	ns	8.48 vs 8.42
HPSP, YPSP vs HPSP+YPSP	2,5 vs 8	1	.005	8.41 vs 8.62
HPSP vs YPSP	2 vs 5	1	.005	8.19 vs 8.62
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	11 vs 14,17,20	1	ns	8.46 vs 8.41
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	14,17 vs 20	1	ns	8.39 vs 8.45
HPSP+BL1 vs YPSP+BL1	14 vs 17	1	.05	8.31 vs 8.46
Plasma Comparisons for Mixed Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	3,6,9 vs 12,15, 18,21	1	ns	8.45 vs 8.41
HPSP, YPSP vs HPSP+YPSP	3,6 vs 9	1	.005	8.37 vs 8.61
HPSP vs YPSP	3 vs 6	1	.005	8.16 vs 8.58
BL1 vs HPSP+BL1, YPSP+ HPSP+YPSP+BL1	12 vs 15,18,21	1	ns	8.39 vs 8.41
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	15,18 vs 21	1	ns	8.39 vs 8.47
HPSP+BL1 vs YPSP+BL1	15 vs 18	1	ns	8.33 vs 8.44

TABLE 18. LEAST SQUARES MEANS FOR MOTILITY OF SPERMATOZOA BEFORE PLACEMENT ON WARBURG (FRESH SAMPLE)

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Total treatments		20		
	Spermatozoa Comparisons			
HS, YS vs HS+YS	1,4,7,10,13,16, 19,2,5,8,11,14, 17,20 vs 3,6,9, 12,15,18,21	1	ns	74.6 vs 75.8
HS vs YS	1,4,7,10,13,16, 19 vs 2,5,8,11, 14,17,20	1	ns	74.6 vs 74.6
	Plasma Comparisons for Hampshire Sperm			
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	1,4,7 vs 10,13, 16,19	1	ns	73.8 vs 75.2
HPSP, YPSP vs HPSP+YPSP	1,4 vs 7	1	ns	72.5 vs 76.3
HPSP vs YPSP	1 vs 4	1	.05	77.5 vs 67.5
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	10 vs 13,16,19	1	ns	71.3 vs 76.5
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	13,16 vs 19	1	ns	76.6 vs 76.3
HPSP+BL1 vs YPSP+BL1	13 vs 16	1	ns	78.1 vs 75.0

TABLE 18 CONTINUED

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Plasma Comparisons for Yorkshire Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	2,5,8 vs 11,14, 17,20	1	ns	72.1 vs 76.4
HPSP, YPSP vs HPSP+YPSP	2,5 vs 8	1	.005	66.9 vs 82.5
HPSP vs YPSP	2 vs 5	1	.005	82.5 vs 51.3
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	11 vs 14,17,20	1	ns	77.5 vs 76.1
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	14,17 vs 20	1	ns	76.0 vs 76.3
HPSP+BL1 vs YPSP+BL1				
Plasma Comparisons for Mixed Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	3,6,9 vs 12,15, 18,21	1	ns	75.0 vs 76.4
HPSP, YPSP vs HPSP+YPSP	3,6 vs 9	1	.05	71.8 vs 81.3
HPSP vs YPSP	3 vs 6	1	.005	80.6 vs 63.1
BL1 vs HPSP+BL1, YPSP+ HPSP+YPSP+BL1	12 vs 15,18,21	1	ns	75.6 vs 76.7
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	15,18 vs 21	1	ns	78.1 vs 73.8
HPSP+BL1 vs YPSP+BL1	15 vs 18	1	ns	80.6 vs 75.6

TABLE 19. LEAST SQUARES MEANS FOR MOTILITY OF SPERMATOZOA AFTER REMOVAL FROM WARBURG (FRESH SAMPLE)

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Total treatments		20		
	Spermatozoa Comparisons			
HS, YS vs HS+YS	1,4,7,10,13,16, 19,2,5,8,11,14, 17,20 vs 3,6,9, 12,15,18,21	1	ns	45.2 vs 46.1
HS vs YS	1,4,7,10,13,16, 19 vs 2,5,8,11, 14,17,20	1	ns	47.8 vs 42.6
	Plasma Comparisons for Hampshire Sperm			
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	1,4,7 vs 10,13, 16,19	1	ns	53.4 vs 43.6
HPSP, YPSP vs HPSP+YPSP	1,4 vs 7	1	ns	48.2 vs 63.8
HPSP vs YPSP	1 vs 4	1	.005	72.5 vs 23.8
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	10 vs 13,16,19	1	ns	36.9 vs 45.9
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	13,16 vs 19	1	ns	48.2 vs 41.3
HPSP+BL1 vs YPSP+BL1	13 vs 16	1	ns	44.4 vs 51.9

TABLE 19 CONTINUED

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Plasma Comparisons for Yorkshire Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	2,5,8 vs 11,14, 17,20	1	ns	40.6 vs 44.1
HPSP, YPSP VS HPSP+YPSP	2,5 vs 8	1	ns	40.0 vs 41.9
HPSP VS YPSP	2 vs 5	1	.005	70.6 vs 9.4
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	11 vs 14,17,20	1	ns	39.4 vs 45.6
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	14,17 vs 20	1	ns	46.3 vs 44.4
HPSP+BL1 vs YPSP+BL1	14 vs 17	1	ns	46.9 vs 45.6
Plasma Comparisons for Mixed Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	3,6,9 vs 12,15, 18,21	1	.05	52.5 vs 41.3
HPSP, YPSP vs HPSP+YPSP	3,6 vs 9	1	ns	53.8 vs 50.0
HPSP vs YPSP	3 vs 6	1	.005	72.5 vs 35.0
BL1 vs HPSP+BL1, YPSP+ HPSP+YPSP+BL1	12 vs 15,18,21	1	ns	37.5 vs 42.5
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	15,18 vs 21	1	ns	43.8 vs 40.0
HPSP+BL1 vs YPSP+BL1	15 vs 18	1	ns	48.8 vs 38.8

Least squares means for percent dead spermatozoa before placement of samples on the Warburg apparatus are presented in table 20. The orthogonal comparison of HS vs YS indicated that HS had a greater ($P<.005$) percent dead spermatozoa (20.97 vs 17.16). All other comparisons were not significant. Percent dead spermatozoa were not different between treatments after the samples were removed from the Warburg apparatus.

The pH of stored semen samples before being placed and after being removed from the Warburg apparatus is shown in tables 21 and 22. The HS had a lower ($P<.005$) pH value (table 21) before incubation than the YS (7.66 vs 7.89). However, this difference was not present (8.34 vs 8.42) after incubation (table 22). The pH values were higher ($P<.005$) when YPSP was used as a diluent for HS, YS and MS than when HPSP was used as a diluent. This difference was present before and after incubation. The comparison of HPSP, YPSP, HPSP + YPSP vs BL1, HPSP + BL1, YPSP + BL1 and HPSP + YPSP + BL1 showed that when HS, YS or MS were diluted with substances containing BL1 a lower ($P<.005$) pH value was found in the stored sample before incubation (table 21). A difference was not found after the 2-hour incubation period. Diluting HS, YS and MS with HPSP + BL1 or YPSP + BL1 resulted in YPSP + BL1 producing a higher (HS, $P<.05$; YS, MS, $P<.005$) pH value and the difference was not present after incubation.

Orthogonal comparisons for motility of stored semen samples are presented in tables 23 (before Warburg) and 24 (after Warburg). Before semen samples were placed on the Warburg a difference ($P<.05$) in

TABLE 20. LEAST SQUARES MEANS FOR PERCENT DEAD SPERMATOZOA BEFORE PLACEMENT IN WARBURG (FRESH SAMPLE)

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Total treatments		20		
	Spermatozoa Comparisons			
HS, YS vs HS+YS	1,4,7,10,13,16, 19,2,5,8,11,14, 17,20 vs 3,6,9, 12,15,18,21	1	ns	19.06 vs 19.07
HS vs YS	1,4,7,10,13,16, 19 vs 2,5,8,11, 14,17,20	1	.005	20.97 vs 17.16
	Plasma Comparisons for Hampshire Sperm			
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	1,4,7 vs 10,13, 16,19	1	ns	22.17 vs 20.08
HPSP, YPSP vs HPSP+YPSP	1,4 vs 7	1	ns	21.75 vs 23.00
HPSP vs YPSP	1 vs 4	1	ns	21.10 vs 22.40
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	10 vs 13,16,19	1	ns	20.50 vs 19.93
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	13,16 vs 19	1	ns	19.60 vs 20.60
HPSP+BL1 vs YPSP+BL1	13 vs 16	1	ns	21.90 vs 17.30

TABLE 20 CONTINUED

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Plasma Comparisons for Yorkshire Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	2,5,8 vs 11,14, 17,20	1	ns	18.67 vs 16.03
HPSP, YPSP vs HPSP+YPSP	2,5 vs 8	1	ns	17.30 vs 21.40
HPSP vs YPSP	2 vs 5	1	ns	19.30 vs 15.30
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	11 vs 14,17,20	1	ns	15.80 vs 16.10
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	14,17 vs 20	1	ns	15.90 vs 16.50
HPSP+BL1 vs YPSP+BL1	14 vs 17	1	ns	17.40 vs 14.40
Plasma Comparisons for Mixed Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	3,6,9 vs 12,15, 18,21	1	ns	19.67 vs 18.63
HPSP, YPSP vs HPSP+YPSP	3,6 vs 9	1	ns	20.55 vs 17.90
HPSP vs YPSP	3 vs 6	1	ns	22.00 vs 19.10
BL1 vs HPSP+BL1, YPSP+ HPSP+YPSP+BL1	12 vs 15,18,21	1	ns	19.10 vs 18.47
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	15,18 vs 21	1	ns	18.75 vs 17.90
HPSP+BL1 vs YPSP+BL1	15 vs 18	1	ns	19.40 vs 18.10

TABLE 21. LEAST SQUARES MEANS FOR SEMEN pH BEFORE PLACEMENT IN WARBURG (STORED SAMPLE)

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Total treatments		20		
	Spermatozoa Comparisons			
HS, YS vs HS+YS	1,4,7,10,13,16, 19,2,5,8,11,14, 17,20 vs 3,6,9, 12,15,18,21	1	ns	7.77 vs 7.81
HS vs YS	1,4,7,10,13,16, 19 vs 2,5,8,11, 14,17,20	1	.005	7.66 vs 7.89
	Plasma Comparisons for Hampshire Sperm			
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	1,4,7 vs 10,13, 16,19	1	.005	7.87 vs 7.50
HPSP, YPSP vs HPSP+YPSP	1,4 vs 7	1	ns	7.85 vs 7.93
HPSP vs YPSP	1 vs 4	1	.005	7.43 vs 8.26
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	10 vs 13,16,19	1	ns	7.46 vs 7.51
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	13,16 vs 19	1	ns	7.51 vs 7.51
HPSP+BL1 vs YPSP+BL1	13 vs 16	1	.05	7.38 vs 7.64

TABLE 21 CONTINUED

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Plasma Comparisons for Yorkshire Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	2,5,8 vs 11,14, 17,20	1	.005	8.05 vs 7.76
HPSP, YPSP vs HPSP+YPSP	2,5 vs 8	1	ns	8.00 vs 8.16
HPSP vs YPSP	2 vs 5	1	.005	7.42 vs 8.58
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	11 vs 14,17,20	1	ns	7.70 vs 7.78
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	14,17 vs 20	1	ns	7.77 vs 7.81
HPSP+BL1 vs YPSP+BL1	14 vs 17	1	.005	7.51 vs 8.02
Plasma Comparisons for Mixed Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	3,6,9 vs 12,15, 18,21	1	.005	7.98 vs 7.68
HPSP, YPSP vs HPSP+YPSP	3,6 vs 9	1	ns	7.95 vs 8.05
HPSP vs YPSP	3 vs 6	1	.005	7.42 vs 8.47
BL1 vs HPSP+BL1, YPSP+ HPSP+YPSP+BL1	12 vs 15,18,21	1	ns	7.61 vs 7.70
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	15,18 vs 21	1	ns	7.69 vs 7.73
HPSP+BL1 vs YPSP+BL1	15 vs 18	1	.005	7.46 vs 7.91

TABLE 22. LEAST SQUARES MEANS FOR SEMEN pH AFTER REMOVAL FROM WARBURG (STORED SAMPLE)

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Total treatments		20		
	Spermatozoa Comparisons			
HS, YS vs HS+YS	1,4,7,10,13,16, 19,2,5,8,11,14, 17,20 vs 3,6,9, 12,15,18,21	1	ns	8.38 vs 8.36
HS vs YS	1,4,7,10,13,16, 19 vs 2,5,8,11, 14,17,20	1	ns	8.34 vs 8.42
	Plasma Comparisons for Hampshire Sperm			
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	1,4,7 vs 10,13, 16,19	1	ns	8.36 vs 8.32
HPSP, YPSP vs HPSP+YPSP	1,4 vs 7	1	ns	8.40 vs 8.29
HPSP vs YPSP	1 vs 4	1	.005	8.14 vs 8.65
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	10 vs 13,16,19	1	ns	8.38 vs 8.30
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	13,16 vs 19	1	ns	8.34 vs 8.22
HPSP+BL1 vs YPSP+BL1	13 vs 16	1	ns	8.35 vs 8.32

TABLE 22 CONTINUED

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Plasma Comparisons for Yorkshire Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	2,5,8 vs 11,14, 17,20	1	ns	8.35 vs 8.47
HPSP, YPSP vs HPSP+YPSP	2,5 vs 8	1	ns	8.30 vs 8.45
HPSP vs YPSP	2 vs 5	1	.005	7.96 vs 8.64
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	11 vs 14,17,20	1	ns	8.58 vs 8.43
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	14,17 vs 20	1	ns	8.41 vs 8.48
HPSP+BL1 vs YPSP+BL1	14 vs 17	1	ns	8.38 vs 8.43
Plasma Comparisons for Mixed Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	3,6,9 vs 12,15, 18,21	1	ns	8.33 vs 8.38
HPSP, YPSP vs HPSP+YPSP	3,6 vs 9	1	ns	8.30 vs 8.39
HPSP vs YPSP	3 vs 6	1	.005	7.93 vs 8.66
BL1 vs HPSP+BL1, YPSP+ HPSP+YPSP+BL1	12 vs 15,18,21	1	ns	8.47 vs 8.35
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	15,18 vs 21	1	ns	8.37 vs 8.31
HPSP+BL1 vs YPSP+BL1	15 vs 18	1	ns	8.33 vs 8.41

TABLE 23. LEAST SQUARES MEANS FOR MOTILITY OF SPERMATOZOA BEFORE PLACEMENT ON WARBURG (STORED SAMPLE)

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means (%)
Total treatments		20		
	Spermatozoa Comparisons			
HS, YS vs HS+YS	1,4,7,10,13,16, 19,2,5,8,11,14, 17,20 vs 3,6,9, 12,15,18,21	1	.05	19.4 vs 15.1
HS vs YS	1,4,7,10,13,16, 19 vs 2,5,8,11, 14,17,20	1	.05	22.0 vs 16.8
	Plasma Comparisons for Hampshire Sperm			
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	1,4,7 vs 10,13, 16,19	1	.005	2.1 vs 36.9
HPSP, YPSP vs HPSP+YPSP	1,4 vs 7	1	ns	1.9 vs 2.5
HPSP vs YPSP	1 vs 4	1	ns	3.8 vs 0
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	10 vs 13,16,19	1	.005	53.8 vs 31.3
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	13,16 vs 19	1	ns	30.7 vs 32.5
HPSP+BL1 vs YPSP+BL1	13 vs 16	1	.005	43.8 vs 17.5

TABLE 23 CONTINUED

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means (%)
Plasma Comparisons for Yorkshire Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	2,5,8 vs 11,14, 17,20	1	.005	.8 vs 28.8
HPSP, YPSP vs HPSP+YPSP	2,5 vs 8	1	ns	1.3 vs 0
HPSP vs YPSP	2 vs 5	1	ns	2.5 vs 0
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	11 vs 14,17,20	1	.005	53.1 vs 20.6
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	14,17 vs 20	1	.01	25.7 vs 10.6
HPSP+BL1 vs YPSP+BL1	14 vs 17	1	.005	47.5 vs 3.8
Plasma Comparisons for Mixed Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	3,6,9 vs 12,15, 18,21	1	.005	1.0 vs 25.5
HPSP, YPSP vs HPSP+YPSP	3,6 vs 9	1	ns	1.6 vs 0
HPSP vs YPSP	3 vs 6	1	ns	3.1 vs 0
BL1 vs HPSP+BL1, YPSP+ HPSP+YPSP+BL1	12 vs 15,18,21	1	.05	36.3 vs 21.9
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	15.18 vs 21	1	ns	24.7 vs 16.3
HPSP+BL1 vs YPSP+BL1	15 vs 18	1	.005	43.1 vs 6.3

TABLE 24. LEAST SQUARES MEANS FOR MOTILITY OF SPERMATOZOA AFTER REMOVAL FROM WARBURG (STORED SAMPLE)

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means (%)
Total treatments		20		
	Spermatozoa Comparisons			
HS, YS vs HS+YS	1,4,7,10,13,16, 19,2,5,8,11,14, 17,20 vs 3,6,9, 12,15,18,21	1	ns	28.1 vs 26.1
HS vs YS	1,4,7,10,13,16, 19 vs 2,5,8,11, 14,17,20	1	ns	30.6 vs 25.6
	Plasma Comparisons for Hampshire Sperm			
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	1,4,7 vs 10,13, 16,19	1	.005	20.8 vs 37.9
HPSP, YPSP vs HPSP+YPSP	1,4 vs 7	1	ns	24.7 vs 13.1
HPSP vs YPSP	1 vs 4	1	.005	49.4 vs 0
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	10 vs 13,16,19	1	ns	29.4 vs 40.7
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	13,16 vs 19	1	.05	35.4 vs 51.3
HPSP+BL1 vs YPSP+BL1	13 vs 16	1	.05	44.4 vs 26.3

TABLE 24 CONTINUED

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means (%)
Plasma Comparisons for Yorkshire Sperm				
HPSP, YPSP, HPSP+YPSP vs BLl, HPSP+BLl, YPSP+ BLl, HPSP+YPSP+BLl	2,5,8 vs 11,14, 17,20	1	.005	17.1 vs 32.1
HPSP, YPSP vs HPSP+YPSP	2,5 vs 8	1	.005	24.4 vs 2.5
HPSP vs YPSP	2 vs 5	1	.005	48.0 vs 0
BLl vs HPSP+BLl, YPSP+ BLl, HPSP+YPSP+BLl	11 vs 14,17,20	1	ns	31.9 vs 32.1
HPSP+BLl, YPSP+BLl vs HPSP+YPSP+BLl	14,17 vs 20	1	ns	33.5 vs 29.4
HPSP+BLl vs YPSP+BLl	14 vs 17	1	.05	43.1 vs 23.8
Plasma Comparisons for Mixed Sperm				
HPSP, YPSP, HPSP+YPSP vs BLl, HPSP+BLl, YPSP+ BLl, HPSP+YPSP+BLl	3,6,9 vs 12,15, 18,21	1	.005	17.1 vs 32.8
HPSP, YPSP vs HPSP+YPSP	3,6 vs 9	1	.005	24.1 vs 3.1
HPSP vs YPSP	3 vs 6	1	.005	48.1 vs 0
BLl vs HPSP+BLl, YPSP+ HPSP+YPSP+BLl	12 vs 15,18,21	1	ns	25.0 vs 35.4
HPSP+BLl, YPSP+BLl vs HPSP+YPSP+BLl	15,18 vs 21	1	ns	31.3 vs 43.8
HPSP+BLl vs YPSP+BLl	15 vs 18	1	.05	41.9 vs 20.6

motility was found between homospermic semen and heterospermic semen. Homospermic semen showed more motility than heterospermic semen (19.4 vs 15.1). In comparing the two homospermic samples, the HS was more motile than the YS (22.0 vs 16.8). The difference in motility between the homospermic samples versus heterospermic samples and the HS vs YS did not exist after incubation. The average motility estimates of the previous comparisons were slightly higher after incubation than before incubation (tables 23 and 24). A difference ($P < .005$) in motility occurred in the comparison of treatments containing only plasma versus those containing plasma plus BL 1 as a diluent for HS, YS and MS in both tables 23 and 24. The presence of BL1 caused the spermatozoa to be more motile after storage when warmed in a 38 C water bath (table 23). The comparison involving only BL1 versus plasma plus BL1 as the diluent for HS, YS or MS showed that BL1 produced a higher ($P < .005$) motility estimate in semen samples before incubation (table 23). This same comparison in table 24 was not significant but showed higher motility estimates for HS, YS and MS diluted with plasma plus BL1. Motility estimates were higher ($P < .005$) when diluting HS, YS and MS with HPSP + BL1 than with YPSP + BL1 (table 23). Although not as great, a difference ($P < .05$) did exist after incubation for the comparison HPSP + BL1 vs YPSP + BL1. Prior to incubation the motility of HS, YS and MS diluted with HPSP or YPSP did not have a difference in response to HPSP or YPSP. This was not true after incubation. The utilization of HPSP caused a substantial increase ($P < .005$) in motility for HS (49.4 vs 0), YS (48.0 vs 0) and MS (48.1 vs 0) when compared to

using YPSP (table 24). It appears that the YPSP has some type of component present that decreases motility. In the comparison HPSP, YPSP vs HPSP + YPSP for YS and MS, motility was less ($P < .005$) when the spermatozoa were diluted with HPSP + YPSP.

An increase ($P < .05$) in percent dead spermatozoa before incubation of stored samples occurred when YPSP was used as a diluent for HS, YS and MS as compared to the diluent HPSP (table 25). The comparison HPSP, YPSP vs HPSP + YPSP for YS revealed less ($P < .05$) dead spermatozoa when HPSP + YPSP was used as a diluent.

The only significant differences in dead spermatozoa after incubation were found in the plasma comparisons for MS (table 26). The comparison between plasma only versus plasma plus BLl revealed less ($P < .005$) dead spermatozoa when BLl was present in the diluent (29.17 vs 19.48). After incubation MS diluted with YPSP produced more ($P < .05$) dead spermatozoa than when diluted with HPSP (36.10 vs 27.30). The comparison HPSP, YPSP vs HPSP + YPSP for MS showed a higher ($P < .05$) number of dead spermatozoa from the dilutions made with HPSP or YPSP (31.70 vs 24.10).

A correlation was performed between pH and motility for all treatments combined. The pH of treatments before incubation was negatively ($P < .01$) correlated with motility before incubation in the fresh ($r = -.42$) and stored ($r = -.52$) samples. A negative ($P < .01$) correlation existed between pH before incubation and motility after incubation in the fresh ($r = -.53$) and stored ($r = -.70$) samples. Motility after incubation was negatively ($P < .01$) correlated in fresh

TABLE 25. LEAST SQUARES MEANS FOR PERCENT DEAD SPERMATOZOA BEFORE PLACEMENT IN WARBURG (STORED SAMPLE)

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Total treatments		20		
	Spermatozoa Comparisons			
HS, YS vs HS+YS	1,4,7,10,13,16, 19,2,5,8,11,14, 17,20 vs 3,6,9, 12,15,18,21	1	ns	17.91 vs 18.79
HS vs YS	1,4,7,10,13,16, 19 vs 2,5,8,11, 14,17,20	1	ns	18.16 vs 17.66
	Plasma Comparisons for Hampshire Sperm			
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	1,4,7 vs 10,13, 16,19	1	ns	21.87 vs 17.30
HPSP, YPSP vs HPSP+YPSP	1,4 vs 7	1	ns	23.35 vs 18.00
HPSP vs YPSP	1 vs 4	1	.01	16.90 vs 23.00
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	10 vs 13,16,19	1	ns	19.50 vs 16.57
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	13,16 vs 19	1	ns	17.30 vs 15.10
HPSP+BL1 vs YPSP+BL1	13 vs 16	1	ns	16.80 vs 17.80

TABLE 25 CONTINUED

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Plasma Comparisons for Yorkshire Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	2,5,8 vs 11,14, 17,20	1	ns	15.30 vs 14.50
HPSP, YPSP vs HPSP+YPSP	2,5 vs 8	1	.05	23.35 vs 18.90
HPSP vs YPSP	2 vs 5	1	.005	19.70 vs 27.00
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	11 vs 14,17,20	1	ns	13.80 vs 14.73
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	14,17 vs 20	1	ns	14.40 vs 15.40
HPSP+BL1 vs YPSP+BL1	14 vs 17	1	ns	15.80 vs 13.00
Plasma Comparisons for Mixed Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	3,6,9 vs 12,15,	1	ns	19.80 vs 18.03
HPSP, YPSP vs HPSP+YPSP	3,6 vs 9	1	ns	20.40 vs 18.60
HPSP vs YPSP	3 vs 6	1	.005	16.40 vs 24.40
BL1 vs HPSP+BL1, YPSP+ HPSP+YPSP+BL1	12 vs 15,18,21	1	ns	18.50 vs 17.87
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	15.18 vs 21	1	ns	17.80 vs 18.00
HPSP+BL1 vs YPSP+BL1	15 vs 18	1	ns	17.10 vs 18.50

TABLE 26. LEAST SQUARES MEANS FOR PERCENT DEAD SPERMATOZOA AFTER REMOVAL FROM WARBURG (STORED SAMPLE)

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Total treatments		20		
	Spermatozoa Comparisons			
HS, YS vs HS+YS	1,4,7,10,13,16, 19,2,5,8,11,14, 17,20 vs 3,6,9, 12,15,18,21	1	ns	22.03 vs 23.63
HS vs YS	1,4,7,10,13,16, 19 vs 2,5,8,11, 14,17,20	1	ns	21.53 vs 22.53
	Plasma Comparisons for Hampshire Sperm			
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	1,4,7 vs 10,13, 16,19	1	ns	21.77 vs 21.35
HPSP, YPSP vs HPSP+YPSP	1,4 vs 7	1	ns	21.60 vs 22.10
HPSP vs YPSP	1 vs 4	1	ns	21.60 vs 21.60
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	10 vs 13,16,19	1	ns	20.50 vs 21.63
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	13,16 vs 19	1	ns	22.05 vs 20.80
HPSP+BL1 vs YPSP+BL1	13 vs 16	1	ns	22.80 vs 21.30

TABLE 26 CONTINUED

Source of variation for orthogonal comparisons	Treatments involved	df	Level of P	Means
Plasma Comparisons for Yorkshire Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	2,5,8 vs 11,14,	1	ns	23.50 vs 21.80
HPSP, YPSP vs HPSP+YPSP	2,5 vs 8	1	ns	23.80 vs 22.90
HPSP vs YPSP	2 vs 5	1	ns	23.30 vs 24.30
BL1 vs HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	11 vs 14,17,20			
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	14,17 vs 20	1	ns	22.65 vs 22.80
HPSP+BL1 vs YPSP+BL1	14 vs 17	1	ns	24.90 vs 20.40
Plasma Comparisons for Mixed Sperm				
HPSP, YPSP, HPSP+YPSP vs BL1, HPSP+BL1, YPSP+ BL1, HPSP+YPSP+BL1	3,6,9 vs 12,15,	1	.005	29.17 vs 19.48
HPSP, YPSP vs HPSP+YPSP	3,6 vs 9	1	.05	31.70 vs 24.10
HPSP vs YPSP	3 vs 6	1	.05	27.30 vs 36.10
BL1 vs HPSP+BL1, YPSP+ HPSP+YPSP+BL1	12 vs 15,18,21	1	ns	17.10 vs 20.27
HPSP+BL1, YPSP+BL1 vs HPSP+YPSP+BL1	15,18 vs 21	1	ns	19.45 vs 21.90
HPSP+BL1 vs YPSP+BL1	15 vs 18	1	ns	19.80 vs 19.10

(r = -.66) and stored (r = -.67) samples with pH after incubation. These correlations indicate that as pH increases the motility decreases.

General Discussion

Semen characteristics of the boars utilized in this investigation are similar to those reported by other researchers. Semen characteristics did differ between the two boars within each pair in experiments 1 to 3. Based on the findings of Singleton (1968) and Swierstra (1973), these differences could be expected.

Gilts inseminated with heterospermic semen had 4.1 and 3.4 more embryos than gilts bred with straight Yorkshire or Hampshire semen, respectively. This indicates that some type of unknown synergistic effect is present. If a synergistic effect is present, it would be of interest to determine if there was a 50:50 ratio in progeny born or is one boar producing the majority of the offspring. Experimental animals and facilities available did not allow this question to be answered.

It is unfortunate that a significant interaction for treatments by days appeared in the analysis for oxygen consumption. This interaction indicates that the treatments react differently from day to day which makes it impossible to draw definite conclusions. The biological explanation for this interaction is not known. One would expect that if something was present in the semen collected, i.e., urine, it would affect all treatments the same.

When diluting HS, YS or MS with straight YPSP, a higher pH resulted than when straight HPSP was used as a diluent. This was true for fresh and stored samples before and after removal of the samples from the Warburg (tables 16, 17, 21 and 22). Dilution with YPSP produced the highest pH values (range 8.58 to 8.66) after incubation when compared to the pH values of other orthogonal comparisons made. According to Shelby (1966) the diethanolamine mixture should remove bicarbonate from the semen until an equilibrium is established between the carbon dioxide in the atmosphere and the bicarbonate in the semen. In a 1% carbon dioxide atmosphere the pH at this equilibrium point is 8.40 and is low enough to prolong the motility and oxygen consumption of boar semen. It is evident from this study that diethanolamine mixture was not capable of maintaining the pH at 8.40 when straight YPSP was used as a diluent for HS, YS or MS. After incubation in both fresh and stored samples, the treatments containing straight YPSP as the diluent had higher pH values, lower motility estimates and decreased oxygen consumption. The explanation for the difference between HPSP and YPSP is not known.

An interesting result for motility estimates on HS, YS or MS diluted with HPSP or YPSP was found after stored samples had been incubated for 2 hours (tables 23 and 24). Estimates made on stored samples (table 23) that were placed in a 38 C water bath for 30 minutes indicated very little motility. However, the aliquots removed from the vials and placed in the Warburg flasks prior to warming the vials showed a substantial increase in motility (table 24) for HS, YS and MS

diluted with HPSP. Motility was still zero after incubation in the Warburg when YPSP was used as the diluent for HS, YS and MS.

The comparisons involving BL1 vs HPSP + BL1, YPSP + BL1 and HPSP + YPSP + BL1 (table 19) indicated ($P < .05$) that motility is less after incubation of fresh semen. The same results were found for stored semen after incubation (table 24). While estimating spermatozoa motility after storage, it was found that spermatozoa diluted with BL1 had a "vibratory" type of motility. It appeared that the protoplasmic membrane surrounding the head had undergone changes which resulted in the spermatozoon head becoming "stuck" to the microscope slide. This phenomenon has also been found by V. G. Pursel (personal communication).

After storage it was generally found that YPSP produced a higher rate of dead spermatozoa when used as a diluent for HS, YS and MS.

In the fresh sample (table 13) BL1 tended ($P > .05$) to reduce oxygen consumption. In the stored semen samples the effect of BL1 on oxygen consumption was variable. To this author's knowledge, no research has been conducted to study the effect of BL1 on oxygen consumption.

In general, conclusions from the research reported herein can be summarized as follows:

1. Pregnancy rate in gilts was not enhanced due to heterospermic insemination.
2. Gilts inseminated with heterospermic semen had more embryos present at slaughter than gilts bred with homospermic semen.

3. When YPSP was used by itself as a diluent for HS, YS and MS, it caused a decrease in motility and an increase in pH and number of dead spermatozoa.
4. Storage generally resulted in a decrease in pH, motility and oxygen consumption.

SUMMARY

The objectives of this investigation were to (1) determine if there is a difference in pregnancy rate and litter size in gilts between heterospermic and homospermic insemination, (2) study the effect of mixing various fresh semen components involving spermatozoa in the sperm-rich fraction, seminal plasma from the centrifuged post-sperm fraction and Beltsville Ll Extender on oxygen consumption, pH, motility and percent dead spermatozoa and (3) study the effect of storage (54 hours) on oxygen consumption, pH, motility and percent dead spermatozoa in the various combinations.

Chi-square analysis indicated no difference ($P > .05$) in pregnancy rate between gilts bred with Yorkshire semen (43%), Hampshire semen (33%) or mixed semen (50%). Only one Yorkshire and one Hampshire boar were used in the pregnancy rate and litter size study. The number of embryos present at slaughter for the gilts bred with mixed semen (9.8) was greater ($P < .05$) than the number for Yorkshire semen (5.7) and Hampshire semen (6.4).

Semen characteristics for the boars used in these investigations (experiments 1 to 3) were significantly different.

One Yorkshire and one Hampshire boar were used in studying the effects of mixing the various semen components on oxygen consumption, pH, motility and dead spermatozoa in the laboratory. When analyzing the data on oxygen consumption, an interaction ($P < .005$) occurred between treatments and days. Therefore, definite conclusions could not

be drawn about the main effect of treatments. Linear regression lines for accumulative microliters of oxygen consumed between fresh and stored semen were different ($P<.01$). Fresh and stored semen produced different ($P<.05$) linear regression lines for microliters of oxygen taken up per interval.

Higher ($P<.005$) pH values and lower ($P<.05$) motility estimates were recorded in fresh and stored semen samples when Hampshire sperm-rich, Yorkshire sperm-rich and mixed sperm-rich were diluted with centrifuged Yorkshire post-sperm plasma as compared to centrifuged Hampshire post-sperm plasma. Correlation coefficients ($P<.01$) for pH and motility indicated that as pH increases the motility decreases.

Dead spermatozoa were generally greatest when Yorkshire post-sperm plasma was used as the only diluent as compared to the Hampshire post-sperm plasma.

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APPENDIX A

Preparation of Live-Dead and Rose-Bengal Staining Solutions

Live-Dead	Rose-Bengal
1% Eosin B	3 g powdered Rose-Bengal
5% nigrosine	1 ml Formalin (40%)
2.5% sodium citrate dihydrate	99 ml distilled water
91.5% distilled water	

APPENDIX B

Procedure for Preparing Treatments

Hampshire

1. Volume
   a. Sperm-rich _____ ml
   b. Post-sperm _____ ml
2. Motility (PFM) _____ %
3. Concentration _____ (10^8/ml)
4. pH
   a. Sperm-rich _____
   b. Post-sperm _____
5. Centrifuge post-sperm at 300g for 10 minutes.
6. Remove seminal plasma from post-sperm by decantation.
   _____ ml seminal plasma removed
7. pH of post-sperm after centrifugation _____
8. Determining ml of Hamp sperm-rich to have a total of 12 ml of extended sample with a concentration of 1 x 10^8 spermatozoa per ml in vials 1, 4, 7, 10, 13, 16 and 19.
   Conc. (10^8/ml) Motility
   _____ x _____ x (X) = 12 ml
   X = _____ ml, _____ µl
9. Determining total amount of extender (HPS, YPS, BL1) to add to the ml of Hamp sperm-rich in item 8 to have a concentration of 1 x 10^8 spermatozoa per ml in vials 1, 4, 7, 10, 13, 16 and 19.
   a. Conc. (10^8/ml) Motility ml HSR (item 8)
   $$\frac{\text{\_\_\_\_\_} \times \text{\_\_\_\_\_} \times \text{\_\_\_\_\_}}{1 \times 10^8} - \text{\_\_\_\_\_ ml HSR} = \text{\_\_\_\_\_ ml}$$
   _____ µl
   b. Item 9 (µl) _____ divided by 2 = _____ µl
   c. Item 9 (µl) _____ divided by 3 = _____ µl
10. Microliters of Hamp sperm-rich to place in vials 3, 6, 9, 12, 15, 18 and 21.
    _____ µl HSR (1/2 of item 8)
11. Check to make sure there is enough Hamp sperm-rich.
    a. µl in item 8 _____ x 7 = _____
    b. µl in item 10 _____ x 7 = _____
    Total _____
12. Total amount of extender (HPS, YPS, BL1) to be placed in vials 3, 6, 9, 12, 15, 18 and 21.
    a. µl in item 9a _____ divided by 2 = _____
    b. µl in item 12a _____ divided by 2 = _____
    c. µl in item 12a _____ divided by 3 = _____

Yorkshire

13. Volume
    a. Sperm-rich _____ ml
    b. Post-sperm _____ ml
14. Motility (PFM) _____ %
15. Concentration _____ (10^8/ml)
16. pH
    a. Sperm-rich _____
    b. Post-sperm _____
17. Centrifuge post-sperm at 300g for 10 minutes.
18. Remove seminal plasma from post-sperm by decantation.
    _____ ml seminal plasma removed
19. pH of post-sperm after centrifugation _____
20. Determining ml of York sperm-rich to have a total of 12 ml of extended sample with a concentration of 1 x 10^8 spermatozoa per ml in vials 2, 5, 8, 11, 14, 17 and 20.
    Conc. (10^8/ml) Motility
    _____ x _____ x (X) = 12 ml
    X = _____ ml, _____ µl
21. Determining total amount of extender (HPS, YPS, BL1) to add to the ml of York sperm-rich in item 20 to have a concentration of 1 x 10^8 spermatozoa per ml in vials 2, 5, 8, 11, 14, 17 and 20.
    a. Conc. (10^8/ml) Motility ml YSR (item 20)
    $$\frac{\text{\_\_\_\_\_} \times \text{\_\_\_\_\_} \times \text{\_\_\_\_\_ ml YSR}}{1 \times 10^8} - \text{\_\_\_\_\_} = \text{\_\_\_\_\_ ml}$$
    _____ µl
    b. Item 21a (µl) _____ divided by 2 = _____ µl
    c. Item 21a (µl) _____ divided by 3 = _____ µl
22. Microliters of York sperm-rich to place in vials 3, 6, 9, 12, 15, 18 and 21.
    _____ µl YSR (1/2 of item 20)
23. Check to make sure there is enough York sperm-rich.
    a. µl in item 20 _____ x 7 = _____
    b. µl in item 22 _____ x 7 = _____
24. Total amount of extender (HPS, YPS, BL1) to be placed in vials 3, 6, 9, 12, 15, 18 and 21.
    a. µl in item 21a _____ divided by 2 = _____
    b. µl in item 24a _____ divided by 2 = _____
    c. µl in item 24a _____ divided by 3 = _____
25. Check to make sure there is enough Hamp and York post-seminal plasma.
    a. µl in item 9a _____ x 1 = _____
    b. µl in item 12a _____ x 5 = _____
    c. µl in item 21a _____ x 1 = _____
    d. µl in item 24a _____ x 5 = _____
    Total _____

26. Preparing treatment vials.

Treatment number		
1		____ µl Hamp plasma (item 9a) ____ µl Hamp sperm (item 8) ____ Total
2		____ µl Hamp plasma (item 21a) ____ µl York sperm (item 20) ____ Total
3	12a ____ + 24a ____ =	____ µl Hamp plasma ____ µl Hamp sperm (item 10) ____ µl York sperm (item 22) ____ Total
4		____ µl York plasma (item 9a) ____ µl Hamp sperm (item 8) ____ Total
5		____ µl York plasma (item 21a) ____ µl York sperm (item 20) ____ Total
6	12a ____ + 24a ____ =	____ µl York plasma ____ µl Hamp sperm (item 10) ____ µl York sperm (item 22) ____ Total
7		____ µl Hamp plasma (item 12a) ____ µl York plasma (item 12a) ____ µl Hamp sperm (item 8) ____ Total
8		____ µl Hamp plasma (item 24a) ____ µl York plasma (item 24a) ____ µl York sperm (item 20) ____ Total
9	12b ____ + 24b ____ =	____ µl Hamp plasma ____ + ____ = ____ µl York plasma ____ µl Hamp sperm (item 10) ____ µl York sperm (item 22) ____ Total
10		____ µl Beltsville (item 9a) ____ µl Hamp sperm (item 8) ____ Total

Treatment number				
11				____ µl Beltsville (item 21a) ____ µl York sperm (item 20) ____ Total
	12a		24a	
12	____	+	____ =	____ µl Beltsville ____ µl Hamp sperm (item 10) ____ µl York sperm (item 22) ____ Total
13				____ µl Beltsville (item 12a) ____ µl Hamp plasma (item 12a) ____ µl Hamp sperm (item 8) ____ Total
14				____ µl Beltsville (item 24a) ____ µl Hamp plasma (item 24a) ____ µl York sperm (item 20) ____ Total
	12b		24b	
15	____	+	____ =	____ µl Beltsville
	____	+	____ =	____ µl Hamp plasma ____ µl Hamp sperm (item 10) ____ µl York sperm (item 22) ____ Total
16				____ µl Beltsville (item 12a) ____ µl York plasma (item 12a) ____ µl Hamp sperm (item 8) ____ Total
17				____ µl Beltsville (item 24a) ____ µl York plasma (item 24a) ____ µl York sperm (item 20) ____ Total
	12b		24b	
18	____	+	____ =	____ µl Beltsville
	____	+	____ =	____ µl York plasma ____ µl Hamp sperm (item 10) ____ µl York sperm (item 22) ____ Total
19				____ µl Beltsville (item 9c) ____ µl Hamp plasma (item 9c) ____ µl York plasma (item 9c) ____ µl Hamp sperm (item 8) ____ Total

Treatment number				
20			____	µl Beltsville (item 21c)
			____	µl Hamp plasma (item 21c)
			____	µl York plasma (item 21c)
			____	µl York sperm (item 20)
			____	Total
	12c	24c		
21	____	+ ____ =	____	µl Beltsville
	____	+ ____ =	____	µl Hamp plasma
	____	+ ____ =	____	µl York plasma
			____	µl Hamp sperm (item 10)
			____	µl York sperm (item 22)
			____	Total