AN ABSTRACT OF THE THESIS OF

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Title THE PH OF BULL SEMEN AND THE VAGINA OF COWS AS RELATED TO FERTILITY

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The purpose of the experimental work reported in this thesis was to determine whether hydrogen-ion concentration of the reproductive tract of the cow and the semen of the bull are factors influencing conception. Also whether pH of semen is a significant measure of semen quality was studied.

In making a total of 145 *in vivo* vaginal pH determinations representing 66 different animals, it was found that the majority of the determinations were in the pH range of 6.70 to 6.99.

A comparison of 72 determinations of animals receiving four or more services before conceiving and classed as difficult breeders with 28 determinations of animals requiring three services or less per conception and classed as normal was made. There was a tendency for the pH values of the difficult breeding group to be slightly more alkaline than the normals, but the difference was not significant.

It was found in comparing the pH values of pregnant and open cows that the values for the pregnant cows were slightly more alkaline.

A group of eight heifers carried on a vitamin A deficient diet showed a more alkaline condition of the genital tract than did the animals on a normal ration. Of the normal animals, 61.2 per cent of the determinations were between pH 6.40 and 6.69 whereas of the low vitamin A group, 60.0 per cent of the determinations were in the range of 6.70 to 6.99.

In determining the pH of fresh undiluted semen from 27 different bulls over a period of 14 months, a range of from 6.15 to 8.05 with an average of 6.73 was found.

In comparing initial pH with volume of ejaculate a general trend existed for increasingly greater percentages of the determinations to fall in the pH range of 6.50 to 6.80 as the volume of the ejaculates increased.

When comparing motility with pH a direct relationship was found to exist in that as the motility declined so also did the acidity.

On comparing the concentration of semen with pH, the same direct relationship was found as existed with motility and pH. Acidity decreased with lowered concentration. The average pH values for the samples in the different concentration classifications were: excellent motility pH 6.65; good plus, 6.73; good, 6.81; fair 6.94; and poor, 7.10.

A variation in pH of samples for various months was shown. The lowest pH values occurred in December and January and the high in April and May. A distinct seasonal difference between the winter and summer months existed.

The average pH for first ejaculates was found to be slightly more acid than for second ejaculates.

Age of bull appears to have some effect on semen pH as was demonstrated by an average pH of 6.79 for 150 samples from bulls under two years of age and 6.70 for 131 samples from bulls two years of age and older.

In studying pH decrease following incubation at 38° C. of fresh undiluted semen it was found that an increase in acidity resulted, the increase being the greatest for those samples having the best motility. This same relationship existed when comparing pH change with concentration of the ejaculate. When pH change following incubation was compared with conception rate a definite inverse relationship was found. The group, composed of 50 per cent of the samples, having the lowest final pH had the highest conception rate. This would indicate that pH change during incubation and a low final pH are measures of fertility of bull semen.

THE PH OF BULL SEMEN AND THE VAGINA OF COWS AS RELATED TO FERTILITY

by

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Introduction

Normal, efficient reproduction is dependent upon the production of highly viable spermatozoa and ova and the maintenance of the genital tracts of both sexes in an optimum condition for fertilization, implantation and fetal development. Unfavorable conditions in the male or female genital tract may make it impossible for perfectly normal germ cells to be fertilized or to continue to develop after fertilization. Lowered fertility results in breeding inefficiency, which means economic loss to the producer.

Breeding efficiency in cattle is a problem long recognized by workers in the field of animal production the world over. It has often been observed by individuals concerned that some cows fail to conceive when mated with sexually potent and fertile bulls. These cows are apparently normal, showing normal estrual cycles and no recognizable genital pathological abnormalties. It is frequently observed that often such cows have conceived when bred shortly following douching of the vagina with weak solutions of sodium bicarbonate.

Through these observations the opinion among some

cattlemen and veterinarians has developed that the vaginal conditions are in some unknown way, deleterious to normal spermatozoa. There is also the hypothesis that the hydrogen-ion concentration of the vagina and its secretions varies from the normal optimum for spermatozoal survival from time to time after the estrual cycle and for this reason conception does not take place.

If losses due to breeding inefficiency in cattle are to be reduced, it is necessary to have some means of diagnosing the level of fertility of the bulls. At present there are several tests used, but only the long-time tests such as per cent non-returns of animals bred or number of services per conception have proved accurate to any large degree. The other method of diagnosing fertility is evaluation of the semen according to motility and concentration which are largely subjective tests. The present need is to develop a method or methods of evaluating semen on the basis of metabolic activity of the spermatozoa.

Whatever the test, it must be fairly easily applicable without the use of complicated equipment. It must be rapid and accurate so that semen may be more accurately and more economically screened to the end that only semen of acceptable quality may be used, thus insuring a relatively high and economical conception rate.

Review of Literature

1. Hydrogen-ion concentration of the genital tract of the cow.

(1) Normal pH of the genital tract of the cow.

It is difficult to say just what should be considered a normal pH for the genital tract of the cow. Different workers and different methods used in making determinations have resulted in a wide variety of pH means and ranges for different groups of cows studied.

Woodman and Hammond (39) studied vaginal and cervical mucus in 30 slaughtered cows and in no case was an acid reaction obtained with litmus paper. The cervical mucus showed a feeble alkaline reaction, whereas the vaginal mucus showed a stronger alkaline reaction.

Asdell, et al (7) in making 32 determinations in six sterile cows found only one case in which the pH was below 7.0 and in that case the acidity was very slight.

The mean *in vivo* pH of cervical and vaginal mucus in work by Herman and Horton (16) was found to be 7.23 during estrus and ranged from 7.25 to 7.39 during non-estrus.

It was found by Lardy, et al (21) that during estrus cervical mucus had a pH of 8.3 as determined

in vitro with the Coleman glass electrode apparatus. They found the fluids present in the uterus to have a pH of 6.8. They believe, as do McNutt, et al (24, p. 419), that the flow of alkaline fluid from the cervix during estrus influences the pH of the vagina.

Perhaps one of the best pieces of work done along this line was that of Dougherty's (12) which represented *in vivo* determinations on 400 cows from 15 different herds. For making his determinations, Dougherty used a specially constructed silver-silver chloride electrode with a standard Beckman pH meter. The readings were taken at the anterior part of the vagina or near the external os of the cervix.

In this study the highest reading obtained was pH 8.00 and the lowest was 5.52. Of the readings 4.75 per cent varied between 5.52 and 6.00, 29.0 per cent between 6.01 and 6.50, 44.75 per cent between 6.51 and 7.00, 19.75 per cent between 7.01 and 7.50 and 1.75 per cent between 7.51 and 8.00.

Work done by Brown (8) using the Dougherty electrode assembly on 807 *in vivo* determinations resulted in 86 per cent of the readings between pH 6.50 and 7.49, 48.33 falling below 7.00 and 51.66 were above 7.00

Lohmann and Ellenberger (22, p. 47) found 945 *in vivo* determinations to have a mean of 6.87 ranging

from 6.10 to 7.90. They also made a study of 35 paired *in vivo* and *in vitro* determinations which showed the *in vivo* determinations to be lower than those made *in vitro*, but the extent of the difference was not uniform. A total of 676 *in vitro* pH determinations were made by Roark and Herman (31, pp.25-26). The highest reading was 8.30, the lowest 6.18, with 63 per cent falling between 6.90 and 7.30. These workers also made a comparison of *in vivo* with *in vitro* readings. They found that the *in vivo* readings were lower than the *in vitro* readings, the extent of the difference not being uniform. The *in vitro* pH values averaged 7.45 or 0.88 above the average of the *in vivo* readings. The range in difference between the two methods was from 0.40 to 1.33 pH units.

Sedgwick, et al (35, p.171) made *in vitro* determinations on 30 cows and found the reaction to be alkaline with the exception of two isolated cases. The pH range was from 7.00 to 8.90 with an average of 7.900.

(2) Changes in pH during the estrual cycle and pregnancy.

Brown (8, p.104) states that, "very little variation in pH was found during the estrual cycle, however, a tendency for the pH value to decline at time of heat was found."

Dougherty (12, p.218) reports six cows in heat

at the time pH determinations were made ranging from 6.72 to 7.08, five of them falling below 7.00.

Lardy, et al (21) found the cervical secretion to be distinctly alkaline during estrus. The average pH was 8.30 as determined in vitro with a Coleman glass electrode.

In the work reported by Lohmann and Ellenberger (22, p.53) in vivo pH determinations were made daily or every few days in vaginas of cows and heifers for a period of 10 weeks. In every instance but one, the lowest pH was recorded on the day of heat or else on the day immediately preceding or following it. There was a marked tendency for the pH readings to decrease just prior to the onset of estrus and to increase following estrus.

It has been reported that a more alkaline condition is present during estrus than during non-estrus (24, p.419 and 31, p.27). Sedgwick, et al (35, pp.169-170) found a decrease in pH immediately following estrus. They theorized that this might be due to a flow of acid cervical mucus into the vagina at this time.

Regarding the vaginal pH during pregnancy, it is stated by Roark and Herman (31, p.26) that without respect to stage of pregnancy a more alkaline condition exists in pregnant cows than in normal or difficult

breeding cows. Also the pH fluctuated less from day to day in pregnant cows. McNutt, et al (24, p.417) report that cows showed a greater pH range in late pregnancy than in early pregnancy. Dougherty (12, p.217) found the average pH for 196 pregnant cows to be 6.58 whereas the average for 139 non-pregnant cows was 6.68. He found the variation to be as great for pregnant as for non-pregnant cows. Herman and Horton (16) found a mean pH of 7.23 during estrus and a range from 7.25 to 7.39 during non-estrus.

(3) Effect of fluids of the reproductive tract of the cow on spermatozoa.

Since the tissue fluids of the female genital tract serve as the media through which the spermatozoa flagellate in their course to the ovum their ultimate effect may be determined largely or entirely by the compatability of these two materials.

Herman and Horton (16) found the penetrability of mucus by spermatozoa to be highest during the first six to ten hours of heat. The volume of mucus was also greatest during this same part of the heat cycle.

(4) Survival time of spermatozoa in the genital tract of the cow and its relation to fertility.

By inseminating numerous cows and heifers at various times during and prior to the end of the heat

period and slaughtering them after allowing sufficient time for ovulation to occur and noting whether fertilization had occurred, Laing (19, p.75) determined how long sperm could survive in the genital tract of the cow. He concluded spermatozoa could survive when deposited from about 16 to 16.5 hours before the end of estrus until ovulation and could reach the Fallopian tube in fertile numbers at any time from then to the end of estrus. When deposited earlier than this the spermatozoa did not survive in sufficient numbers until ovulation.

(5) pH of the genital tract of normal and sterile dairy cows.

Since acidity of the vaginal tract is not infrequently cited as a cause of sterility in dairy cattle, several workers (8, 12, 31, 35) have made studies of and compared the vaginal pH of the apparently normal and sterile or difficult breeding animals. The majority of these studies (8, 12, 35) revealed no correlation between vaginal pH and extent of breeding trouble. It is reported by Roark and Herman (31, p.26) however, that in comparing <u>in vivo</u> pH of normal with difficult breeding cows, the pH of the difficult breeding cows tended to be more acid than that of normal cows.

(6) Daily variations in pH of the genital tract.

In making pH determinations on groups of cows periodically every two to four days throughout the estrual cycle numerous workers (12, p.217, 31, p.27 and 35) have shown that the day-to-day variation is greater for individual cows than the overall variation is between cows. The studies have all shown considerable variation from one reading to the next, some as great as one full pH unit and others showing none. One characteristic trend followed by all of the readings, however, is the increased acidity one to three days prior to estrus with the greatest acidity occurring during estrus, followed by a corresponding change toward alkalinity for the following one to three days.

2. Studies on pH of genital tracts of other mammals.

Dybing (14) found in studying the genital tracts of mares that the pH values in the various sections were: vestibulum 7.90, vagina 7.40, cervical canal 6.90 and uterus 6.70. He found the pH during estrus to be 6.90. Quinlan (26, p.112) reports that in Merino ewes there is a tendency toward increased acidity of the vaginal secretions during estrus and pregnancy and that the fertility was higher with pH measurements below 7.0 than with those above 7.00.

Ransom and Zuckerman (27) in reporting the

results of 35 pH determinations on the vaginal tract of 14 monkeys give an average reading of 6.90, ranging from 5.20 to 8.70.

Sedgwick, et al (35, p.173) state that the pH of the bovine vagina is alkaline in contrast to the adult human vagina which is acid. They further explain that this is not surprising since other bovine body fluids such as saliva and urine are alkaline also; their basis for the conclusion being that the diets of cattle have an alkaline residue while the usual human diet has acid residue.

3. Hydrogen-ion concentration of ejaculated spermatozoa of the bull.

Much experimental work in the field of artificial breeding has been directed toward establishing tests for determining potential fertility of semen samples before they are used for breeding purposes. Many tests have been reported to be associated with fertility and among these are metabolism tests of which hydrogen-ion concentration is one, as pH is one of the more important environmental factors affecting cellular activity.

(1) Normal pH of fresh undilated spermatozoa.

One worker (2, p.299) gave the mean pH of 221 ejaculates from clinically normal bulls to be 6.73, the range being from 6.36 to 7.61. Other workers (11) have

reported the mean pH for 146 first ejaculates to be 6.85 with a range of from 6.18 to 8.16. Laing (18, p. 11) found the mean pH of 15 samples to be 6.72, varying from 6.48 to 6.99. Another report (32) shows the average pH of semen samples of good quality to be 6.65. It is further stated that "those samples of poor quality had an average pH of 7.03." Van Rensburg and Starke (30) give an average pH for bull semen of 6.60 to 6.80. Sergin (36) reports the pH of semen as determined by a potentiometer with quinhydrol electrode to be 6.75. Another author (17, p.436) stated, "the pH value of sperm from healthy bulls is just below the neutral point. Whenever possible, only semen with a pH value between 6.30 and 7.00 should be used, for any alkalescence is proof of contamination." Raps and Cannon (28, p.934) found the average of the mean pH of over 100 first ejaculates representing five different studs to be 6.76. Schneerson (34) noted that the pH of semen from fertile bulls should be below 6.60.

(2) Concentration of spermatozoa and pH.

Anderson (2, p.300) reported a highly significant linear regression of the pH on the concentration of spermatozoa. Other workers (30, 11) found that the lower the initial pH the greater the density of the semen.

Laing (18, p. 11) reports from his work that there did not appear to be any relationship between the initial pH and density of the ejaculate. This is based, however, on only 15 samples.

(3) Motility of spermatozoa and pH.

In comparing the pH with the initial motility of freshly ejaculated semen, the several different workers (30, 11, 2, 22) are quite well in agreement on their findings. Studies reported by Anderson (2, p.300) on 221 ejaculates of semen from clinically normal bulls revealed a highly significant linear regression of the pH of the initial motility of the spermatozoa. Other workers (22, p.53) found a highly significant negative correlation of pH with initial percentage of very progressive motility. Davis and Williams (11) and Van Rensburg and Starke (30) both found that the higher the initial pH, the lower the motility or the lower the initial pH, the higher the motility.

(4) Volume of spermatozoa and pH.

When volume and initial pH of 221 ejaculates were compared by Anderson (2, p.300) a highly significant linear regression of the pH on the volume was found; the more acid the pH, the greater the volume. Other workers (11) also report a highly significant negative relationship existing, thus the higher the pH the

lower the volume.

(5) Fertility of spermatozoa and pH.

Schneerson (34) noted that the pH of semen from fertile bulls should be below 6.60, while Davis and Williams (11) found that the majority of ejaculates from 11 fertile bulls was alkaline in reaction.

Swanson and Herman (38) found that the pH was practically the same for the more fertile bulls (pH 6.47) and bulls of questionable fertility (pH 6.50) and although it was slightly higher for the poor breeders, the correlation between the pH of fresh seman and the conception rate was nonsignificant. Another reviewer (1, p.4) stated "An alkaline reaction is characteristic of sterile bulls. In clinically normal bulls semen of poor quality tends to approach the neutral point or become alkaline...While characteristically the semen of fertile bulls has an acid reaction, the pH range of the semen of fertile bulls may be quite wide, and within this range it is not clear that the pH has any special value apart from its general indication of the other semen properties such as the motility and concentration of spermatozoa."

Dougherty and Ewalt (13) reported little correlation between breeding efficiency and pH of semen.

(6) Changes in semen characteristics following incubation.

The incubation test is based upon the known fact that lactic acid formation occurs during the storage of semen and is accompanied by a decrease in the quantity of glucose, a reduction in motility and an increase in acidity (3, p.70; 4, p.14; 18, p.12; 22, p.53; 29, p.384; 1, p.4). Incubation increases the rate of these reactions allowing measurable differences in shorter times.

The pH drop effected by incubation appears to be a quantitative reaction dependent upon the metabolic activity of the individual spermatozoa and the total number of sperm present. It is an indirect measure of overall metabolism of semen.

According to Anderson (3, p.70) who made a study of 134 ejaculates from seven bulls, a highly significant negative correlation was noted between the change in pH following incubation at 37 degrees for one hour and the number of spermatozoa per c.m.m. and the initial motility in that the greater the decrease in pH the higher the degree of initial motility and the greater the concentration. He further found that the greater the pH decrease, the lower was the initial pH. There were some exceptions to this rule.

Eight ejaculates of the 134 had an initial pH of 7.11 or higher and showed either poor or no motility upon collection. Of these, seven had plus pH change values.

The greater the pH change, the poorer was the motility after incubation. Anderson (4) theorizes that the decrease in pH of the semen after incubation is probably due to the metabolic activity of the spermatozoa.

Of the 134 ejaculates in this study, 17 per cent failed to show a relationship between the pH change after incubation and motility.

In another report by this same worker (4, p.15) he concludes that for a maximum increase in acidity on incubation at 37° C., presence of the following semen characteristics was necessary: a dense sample of activity motile sperm of high metabolic activity, an adequate amount of glucose and conditions favorable for the retention in the semen of any acid products that may be formed. He further states that "the varying buffer capacity of undiluted semen does not seem to be a factor of importance in influencing the final pH of undiluted semen following incubation."

Laing (18, p.11) reported studies similar to those reported by Anderson (4). The samples were

incubated for two hours at 37° C. with both motility and pH readings being made every 15 minutes. Of the 15 samples, three increased in pH under incubation for the first 30 to 45 minutes as much as from 6.63 to 6.91 before a decrease began. Of the remaining 12 samples, a continuous decrease in pH from the initial value occurred upon incubation. The drop in pH was most rapid during the first hour. He also reports that there seemed to be a correlation between the fall in pH and the density, also between the final pH and density. The greater the density, the greater the fall and the lower the final pH.

After two hours incubation, the motility in three samples equalled that on ejaculation, but in all other cases it was reduced or absent. The four samples showing the lowest final pH were all nonmotile at the end of two hours incubation.

Semen samples collected from 19 bulls were incubated and the fall in pH was compared to fertility. In general, the higher the service rate the greater the final pH.

Other workers (22, p.27) also report a significant negative correlation between pH values after cessation of motility of semen incubated at 40° C. and conception rates of portions of the same samples.

Following incubation at 40° C. until all motility had ceased the pH was on the average 1.17 pH units lower than the initial pH. In the cases of samples with very poor initial motility or no motility, the trend was for the pH to increase on incubation or become more alkaline. Along this same line, Romijn (32) reported the storage of sperm at 38° C. to decrease the pH of good semen by about 0.5 pH units during the first hour, but only about 0.2 pH units in the case of poor quality semen.

Reid, et al (29) conducted a study consisting of 203 samples from 11 bulls in which semen was incubated for one hour at 37° C. They reported finding a highly significant positive correlation between pH change of incubated semen and each of the characteristics, concentration, initial motility and viability of spermatozoa. It was also found by these workers that large numbers of abnormal spermatozoa minimized the extent to which pH was changed, which would suggest that these spermatozoa are participating in katabolism very little if at all.

(7) Influence of number of ejaculates upon pH of spermatozoa.

It has been shown that there is a change in pH of successive ejaculates taken at relatively short

intervals (2, 5, and 11).

Davis and Williams (11) reported a mean pH of 6.85 for 146 first ejaculates. In the case of the second ejaculates they found a mean pH of 7.23 while 20 third ejaculate samples had a mean pH of 7.21.

Anderson (5) found a mean pH of 7.68 for three first ejaculates from three bulls considered to be abnormal due to small testes, while the three second ejaculates had a mean of 7.85.

In another report, Anderson (2) showed on 32 occasions where two ejaculates were collected within a few minutes of each other, the first to have an average pH of 6.73 while on the second ejaculates the average was 6.67.

According to Raps and Cannon (28) who made a study of 371 semen samples from 66 bulls in five different studs, the average pH of the second ejaculation from all bulls in all studs was more alkaline than the first ejaculate.

(8) Seasonal effect upon pH of spermatozoa.

Anderson (2) reported no significant monthly variation in the pH of the semen from 221 ejaculates taken over a period of six months. According to other workers (28) reporting a study on 371 samples from 66 bulls taken over a period of 11 months a definite

seasonal fluctuation could be recognized as there were marked variations throughout the 11 month study, February showing the most alkaline (6.95) and the month of May showing the most acid pH (6.25). Erb, et al (15) report in a study using 879 ejaculates from nine bulls the average initial pH of the semen showed little seasonal change either in the same or between different bulls and there were no significant trends. Anderson (6) reported a seasonal variation on a study with nine different bulls.

(9) Management and pH.

It was stated in one report (28) that "A highly significant correlation was found between pH levels of semen samples and the individual bull studs in which they were produced," which would indicate that management is a factor influencing initial pH. The most alkaline pH (mean of 7.13) was produced in the stud having the poorest management and the most acid (mean of 6.57) in the stud following the most desirable management practices.

(10) Semen pH of sterile bulls.

In a study of pH of semen from apparently sterile bulls, Anderson (5) found 30 ejaculates from 15 bulls with epididymitis to have a mean pH of 7.61, with only 6 per cent of the ejaculates having a pH lower than

7.00. He found that the pH of ejaculates collected successively from typical cases of epididymitis became increasingly alkaline. Six ejaculates from three bulls with small testes had a mean pH of 7.73.

Laing (18) reported a comparison of fall in pH following incubation with fertility. He states that "In general, the higher the service rate, the greater the final pH." Lohmann and Ellenberger (22) made a similar comparison and found that those semen samples with a final pH of 7.00 to 7.49 had conception rate of 48 per cent and those from 7.50 to 7.99 had a conception rate of 32 per cent as compared to a conception rate of 62 per cent for those samples with a final pH of from 6.00 to 6.49.

Van Rensburg and Starke (30) state that "When the pH of semen rises to over 7.00 there is usually a decrease or complete cessation of spermatogenesis or a pathological condition in one or both veseculae seminales."

(11) Storage qualities and pH.

It has been recognized (2, 4, 10, and 30) that within limits, the more acid the semen on collection, the better it retained its motility on storage. In contrast to this, rabbit spermatozoa is more sensative to

acidity than alkalinity having as an optimum pH for storage 7.60.

It is concluded that determinations of the initial pH of the semen at the time of collection provides a valuable estimate of the relative potential survival capacity of different ejaculates.

4. Diluters.

The main objectives of a diluting fluid are to increase the volume of the ejaculate and to provide a favorable media for the survival of the sperm.

The following factors must be considered in the preparation of a diluting fluid for bovine sperm: (1) osmotic pressure, (2) pH, (3) buffering capacity, and (4) non-toxicity.

Fresh egg-yolk and citrate or phosphate has been used very extensively as a diluent for bull semen and has given excellent results. One serious disadvantage of the yolk phosphade diluent is the presence of large fat globules which make microscope examination of the diluted semen difficult. It was found that by using egg yolk with citrate instead of phosphate, the fat globules and other material in the yolk were dispersed so that the resulting mixture was clear in appearance (33) thus making microscopic examination much clearer and more accurate.

Egg-yolk contains what is termed "a spermatozoan resistance factor" (23, 25). Studies have shown that this factor in egg-yolk protects the spermatozoa from temperature shock, pH change, osmotic pressure changes, and presence or accumulation of harmful substances (7). This active resistance factor has been isolated (23) and some of its chemical characteristics are, solubility in acetone, alcohol, ether and benzene. A suspension of this active fraction in water has a pH of 3.50, suggestive of its acid character. It gives a water-clear solution in phosphate buffer.

The optimum pH of the diluter for storage was found to be 6.75 and the narrow pH range of 6.70 to 6.80 gave the best preservation of motility. A study of the pH of different egg-yolks used showed a wide variation in pH (6.00 to 6.53) (25).

Materials and Methods

1. Animals

(1) Vaginal studies.

This part of the experiment was carried on with the breeding females in the Oregon State College dairy herd of which about equal numbers are Jerseys and Holsteins. The study was carried on for a period of twelve months.

Both the cows and heifers were fed normal rations in all cases except one group of heifers which were on a vitamin A deficient ration. They will be handled separately in the report. The remainder of the animals received good quality hay, silage and concentrates during the winter months from October to April and irrigated Ladino clover pasture and concentrates during the summer months.

(2) Semen studies.

The semen work was carried on both with the herd sires and groups of experimental bulls at Oregon State College and with bulls used by the Oregon Dairy Breeders Association. The animals ranged in age from one to ten years, and represented three different breeds--Jersey, Holstein, and Guernsey. All of the animals were in good condition throughout the period of study. They

were housed and cared for according to good management practices.

The bulls represented in the part of the study in which influence of season upon the hydrogen-ion concentration of semen was carried on, were confined to indoor pens with access to 10 feet by 60 feet, concrete slab exercise pens during the winter months from October to April and were fed a ration of good quality hay, silage and a grain mix. During the summer months, from May through September, they were turned out on pasture composed of Ladino clover and mixed grasses at least three times each week and in some cases every day. The feeding of grain was continued throughout the pasture season, and hay was fed only in very limited amounts.

2. Equipment

(1) Vaginal studies.

Any animal to be examined was secured in a stall so constructed as to prevent excessive moving about.

A Beckman pH meter was used in this work, using a glass electrode assemblage, as described by Dougherty (12), constructed especially for this purpose.

A glass speculum with a 1.25 inch diameter and 16 inches long was inserted into the vagina until the end was near the cervix. This was done to avoid contaminating the electrodes with secretions from other areas

of the vagina before they reached the cervix at which place the reading was taken.

(2) Semen studies.

All semen was collected by means of the artificial vagina according to the method described by Lambert and McKenzie (20).

The initial motility and concentration of the sperm were examined microscopically (100 x) on a warm stage at 38° C. The pH reading was made with a standard Beckman pH meter using potassium chloride--calomel electrodes.

For incubating semen samples an electric thermostatically controlled warm air incubator set at 38° C. was used.

(3) Sanitation.

All glassware used in handling the semen was washed in a strong washing powder, rinsed with hot tap water and sterilized by dry heat in an electric oven at or above 161° C. for a period of not less than two hours. All rubber goods from the artificial vaginas were washed in the same strong washing powder, thoroughly rinsed in hot tap water and hung up to dry in an enclosed cabinet at room temperature.

Glass speculums were washed and sterilized in the same manner as the other glassware between uses.

The special probe electrode assembly was rinsed between different cows with distilled water followed by immersion in 95 per cent alcohol by that part of the electrode which came in contact with the genital tract of the cow.

(4) Incubation.

A warm air type incubator equipped with an automatic themostat and set at 38° C. was used in the incubation work with the semen.

The semen was examined for concentration, pH, and motility immediately following collection. A 1 ml. portion of the ejaculate was then placed in an 8 ml. pyrex test tube, stoppered and placed in the incubator and held at 38° C. for one hour at which time it was again checked for motility and pH. It was incubated for a second hour and pH and motility were again checked.

Procedure

1. *In vivo* pH determinations of the genital tract of the cow.

Over 140 examinations were made on 66 cows and heifers from the Oregon State College dairy herd plus a few animals in farmers herds in which a certain amount of breeding trouble was being encountered. No selection of cases was made, the animals being placed on the experiment as they came in heat following parturition. A part of the heifers used in the experiment consisted of eight animals which were being carried on a diet low in vitamin A and were at no time turned out on pasture. This group for the most part were following fairly regular estrus cycles, however, at times they were somewhat difficult to detect in heat for seldom did they show the characteristic signs of mounting one another, but instead they did considerable bawling. It was by the bawling, nervous reactions and excessive clear mucous discharge at the vulva that the animals were detected as being in heat.

The practice was to determine the pH about midway during the estrus period on those cows that were checked during estrus. Some of the determinations were made at other times during the estrus cycle than during

the time the animal was in heat.

Two mature cows, one a Holstein, open, and the other a Jersey, pregnant, were examined periodically every other day for a period of 32 days to determine fluctuations during different stages of the estrus cycle and of pregnancy.

In making examinations, the animals were secured in a stanchion that would prevent excessive moving about. The animals were handled with as little excitement and disturbance as possible. In some cases the animals to be examined were moved to a stanchion in the semen collecting room which was in the same building and adjacent to the main laboratory.

The Beckman pH meter and specially designed potassium chloride-calomel electrode were standardized against a standard buffer solution - pH 7.00 before each series of determinations. If more than three animals were examined at one time, the electrode was again checked to see that it was still standardized at pH 7.00. The temperature compensator on the potentiometer was set at 38° C.

After standardization, the electrode was rinsed with distilled water and placed in a solution of 95 per cent alcohol until ready for use.

The area around the vulva of the animal to be

examined was thoroughly washed with soap and water, rinsed with water and wiped dry with paper toweling. The glass speculum was then inserted into the vagina until the end was near the external os of the cervix. The purpose of the speculum was to avoid allowing the electrodes to come in contact with secretions of the different areas of the genital tract as the electrode assembly was inserted. It is known that the various sections of the genital tract of mammals do have different pH readings (14). A pen-type flashlight was used in determining when the lower tip of the speculum was at or near the cervix.

When the speculum was properly inserted, the probe electrode was passed through the speculum until it was felt that the tip was against the cervix. In some cases where the cervix was extremely wide open, the tip of the electrode probably extended into the cervix to a depth of one-half to one inch. After the electrode was properly placed, a period of one and one-half minutes was allowed for the temperatures of the cervix and the electrode to become equal.

Three readings were taken within one and one-half minutes and the average of these taken as the final pH. The electrode and speculum were then withdrawn, and the electrode was wiped clean with a moist paper towel,

washed in distilled water and placed in 95 per cent alcohol. The speculum was washed with a strong washing powder, rinsed and sterilized with dry heat.

All animals that were to be inseminated were artificially served immediately following the pH determination.

2. Laboratory procedure with semen studies.

All semen studied was collected with the artificial vagina following the method of Lambert and McKenzie (20). When making collections, bulls mounted either a dummy cow or another bull. In most cases the bulls that were used in the study were collected from every six days. However, there were a few animals that were on different schedules which varied from collecting once every five days to once every two weeks. It was the general practice to "tease" and stimulate the bull at the dummy or other bull for about five minutes so that when the collection was made it would be a complete ejaculate. Following a collection, the semen was taken into the laboratory, which adjoined the collection room, and processed immediately.

(1) Volume of semen per ejaculate (ml.).

The volume of semen per ejaculate was measured by the use of graduated collection tubes.

(2) Concentration of semen.

The measure of concentration was purely a subjective test, the density being observed under the microscope (100 x) also in the test tube and as a sample of it was poured from the test tube. The concentration was graded from 0 to 10 where 10 denoted the greatest density.

(3) Motility of spermatozoa in fresh semen.

Within not more than three minutes after the semen was collected, a drop of the ejaculate was placed on a glass slide heated by an electric warming stage and examined for degree of motility. This was determined by the presence and rate of movement of the swirls present in the microscope field. The motility was graded from 0 to 10 where 10 denoted the best motility. As in concentration the grades were later regrouped to form five grades--excellent, good plus, good, fair and poor. Nine and ten represented excellent, eight and nine represented good plus, five and six good, three and four fair, and one and two poor.

(4) Semen pH determination.

In the case of the studies carried on in cooperation with the Oregon Dairy Breeders Association, only those samples which had a surplus above the amount required for diluting and shipping were used. In some cases it was necessary to take a second ejaculate from

the same bull to obtain sufficient volume to meet the shipping needs. In these events, the sample examined was a portion of the two ejaculates after being combined and mixed.

For all pH eterminations, a 1 ml. portion of the ejaculate being examined was placed in the small glass container supplied with Beckman pH meters for use with the two-inch electrodes. The pH meter having been standardized against a standard buffer - pH 7.00, the reading was taken of the 1 ml. sample of semen. A series of three readings were made for a period of one minute to insure against an incorrect reading due to "drifting" and the average used.

3. Semen fertility determination.

The actual fertility of the semen was determined from 45 to 60 day non-return rates on animals inseminated in the field by technicians of the Oregon Dairy Breeders Association. Such small numbers of animals were inseminated with semen from the bulls in the Oregon State College herd that no accurate estimate of fertility was possible.

4. Incubation of semen.

For incubation a 1 ml. sample of semen was placed in an 8 ml, pyrex test tube, stoppered and placed in an upright position in an electric thermostatically

regulated incubator which was set at 38° C.

After one hour the semen sample was taken from the incubator, the motility checked with a small drop and the remainder poured into the 5 ml. cup for a pH determination. The small 5 ml. cup was kept in the incubator prior to use to avoid any sudden temperature change in pouring the semen from the test tube. Following the pH determination, the sample was again returned to the incubator for a second period of one hour. The total time required to make the motility and pH determinations at the end of the first hour was not over three minutes.

At the end of the second hour of incubation, pH and motility were determined again, which completed the examination of the portion of the ejaculate.

Results and Discussion

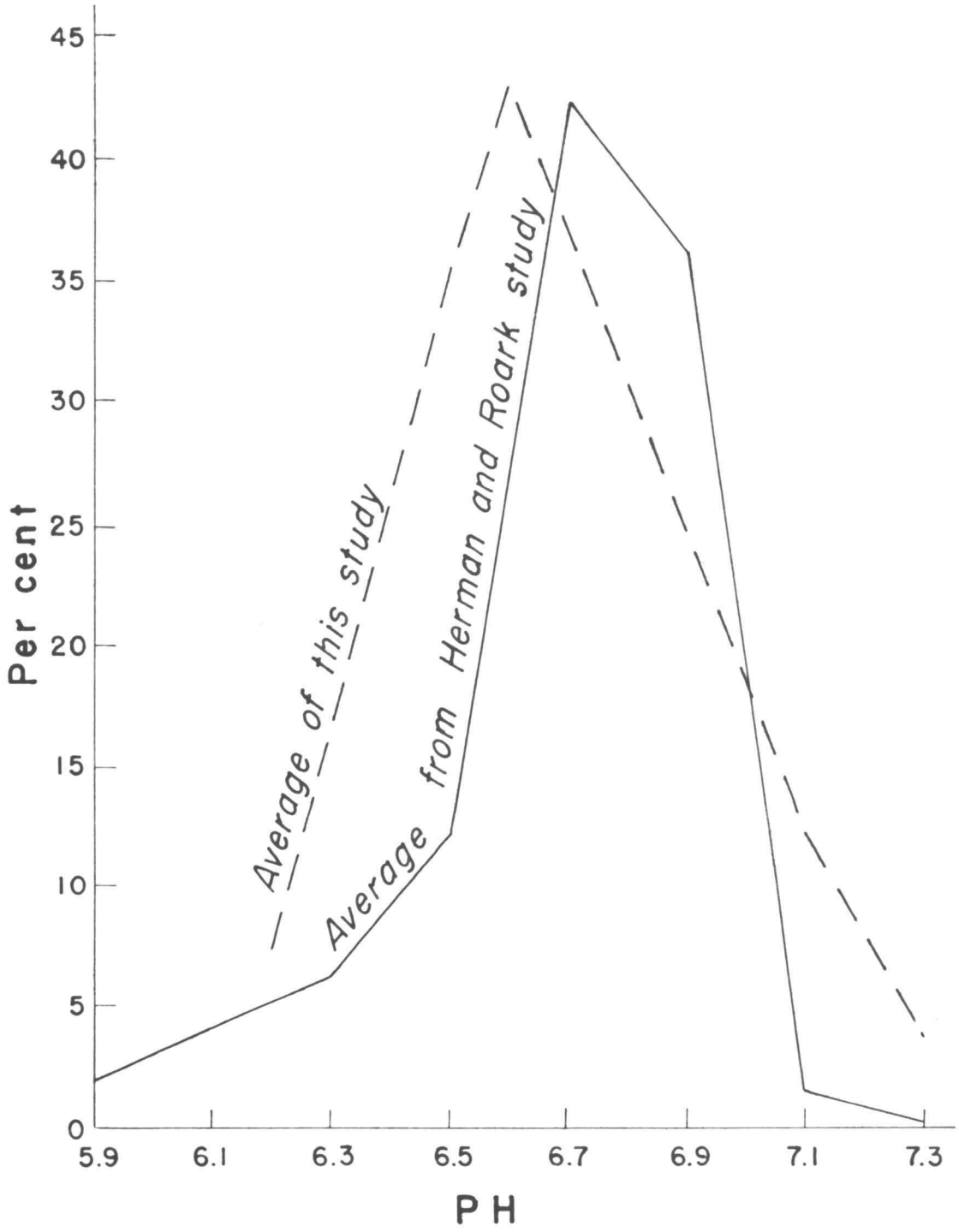
1. Vaginal studies.

(1) Normal range of *in vivo* pH values.

A total of 145 *in vivo* pH determinations was made during different stages of the estrual cycle and pregnancy. The highest pH reading obtained was 7.81 and the lowest 5.73, the average being 6.83. Of the pH values 2.1 per cent varied between 5.73 and 6.08, 12.5 per cent between 6.10 and 6.39, 26.4 per cent between 6.40 and 6.69, 31.3 per cent between 6.70 and 6.99, 20.0 per cent between 7.00 and 7.29, 5.5 per cent between 7.30 and 7.59 and 2.1 per cent between 7.60 and 7.81. There is a tendency for the pH values to group themselves around pH 6.70 and 6.99 which is only one-tenth of a pH unit nearer neutrality than the range in which the pH values given by Dougherty (12) were grouped. In comparing the distribution curve in Figure 1 of 28 determinations made on normal cows in this study with normal cows as determined by Roark and Herman (31, p.27), it will be noted that as a whole these determinations are slightly more acid than those of the other workers. A little greater variation is shown in the distribution of the readings in this study.

(2) Difficult breeders compared to normal animals.

Figure I:- AVERAGE DETERMINATION FOR THIS STUDY AND THAT OF ROARK AND HERMAN



Vaginal pH values of 72 cows classed as difficult breeders varied from 5.75 to 7.70, a variation just slightly greater than that found by Dougherty (12, p.217) in 50 cows classed as difficult breeders. All cows and heifers having four or more services, whether pregnant or not, were classed as difficult breeders. All pH determinations were made at the time the animals were in estrus and just prior to insemination. The pH distribution of the two groups is shown in Table 1. No relation was noted between breeding efficiency and vaginal pH.

Table 1. PH RANGE OF NORMAL AND DIFFICULT BREEDING ANIMALS

pH ranges	Difficult breeders		Normal breeders	
	No. of Samples	Per cent	No. of Samples	Per cent
5.7 - 6.0	3	4.2	0	0
6.1 - 6.3	12	16.7	2	7.1
6.4 - 6.6	16	22.2	10	35.6
6.7 - 6.9	21	29.2	12	43.0
7.0 - 7.2	13	18.1	3	11.7
7.3 - 7.5	5	6.9	1	3.6
7.6 - 7.8	2	2.8	0	0
Total	72		28	

(3) pH values of heifers and cows.

A comparison of pH values of cows was made with those of heifers in an attempt to show any age difference. The range and grouping was very nearly the same for both age groups as shown in Table 2 which would indicate that

no significant difference exists. This again is a comparison of determinations made during estrus.

Table 2. PH VALUES OF NORMAL HEIFERS AND COWS

pH range	Heifers		Cows	
	No. of Animals	Per cent	No. of Animals	Per cent
5.7 - 6.0	0	0	2	3.3
6.1 - 6.3	2	11.1	11	18.4
6.4 - 6.6	5	27.7	17	28.5
6.7 - 6.9	6	33.4	20	33.3
7.0 - 7.2	3	16.7	7	11.7
7.3 - 7.5	2	11.1	2	3.3
7.6 - 7.8	0	0	1	1.7
Total	18		60	

(4) pH value at time of estrus of cows conceiving and those returning for further service.

The vaginal pH determinations of 18 animals made just preceding inseminations to which these cows became pregnant were compared to 84 pH determinations made just preceding inseminations which did not result in pregnancy. The average pH for the group that conceived was 6.69 as compared to 6.73 for those that did not conceive. The distribution range for the two groups of readings is about the same. However, in the first group 33.3 per cent of the determinations fell in the pH range of 6.40 to 6.69, and only 23.6 per cent of the second group fell in this pH range. Only 22.3 per cent of the pregnant group fell in the 6.70 to 6.99 range and 34.6

per cent of the second group were in this range. This is shown graphically in Figure 2.

(5) pH values of pregnant and open cows.

The vaginal pH of pregnant cows, without regard to stage of pregnancy, was more alkaline than that of open cows. The results are given in Table 3 from which it will be noted that 59.2 per cent of the pH values of the pregnant cows and 52.1 per cent of the open cows fell between 6.70 and 7.29. The ph values also varied less for the group of pregnant cows.

Table 3. PH VALUES OF PREGNANT AND OPEN COWS

pH range	Out of heat (open)		Pregnant	
	No.	Per cent	No.	Per cent
5.7 - 6.0	0	0	0	0
6.1 - 6.3	2	8.7	2	9.0
6.4 - 6.6	7	30.4	6	27.3
6.7 - 6.9	7	30.4	5	22.8
7.0 - 7.2	5	21.7	8	36.4
7.3 - 7.5	1	4.4	1	4.5
7.6 - 7.8	1	4.4	0	0
Total	23		22	

(6) pH values during estrus and non-estrus.

In comparing 99 vaginal pH determinations made at the time the animals were in heat with 23 determinations made in open animals out of heat, no significant difference is noted (Table 4).

Figure 2:- DETERMINATIONS FOR SERVICES TO WHICH COWS CONCEIVED AND FAILED TO CONCEIVE

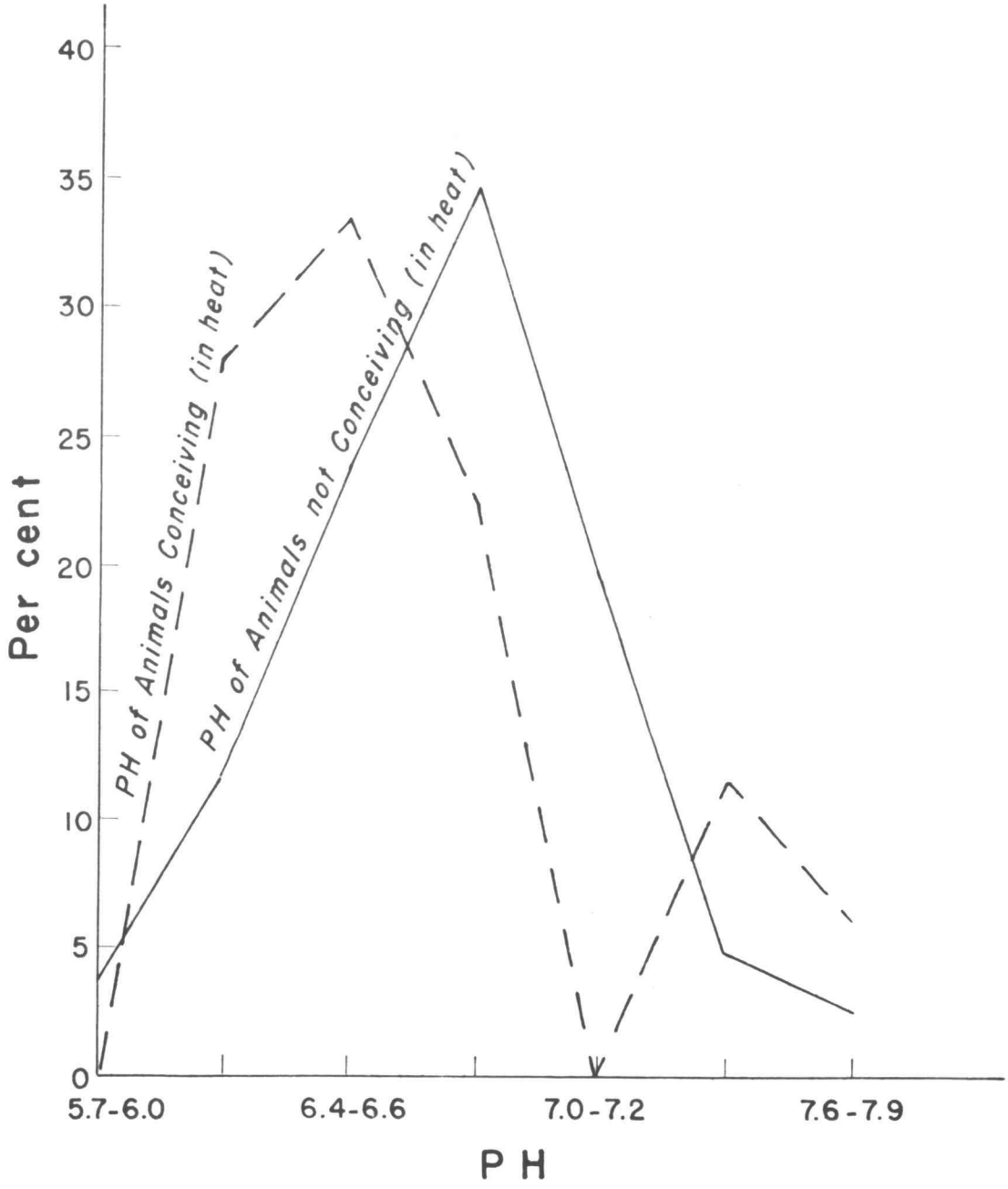


Table 4. DETERMINATIONS OF COWS IN HEAT AND OUT OF HEAT

pH range	In heat		Not in heat (open)	
	Number	Per cent	Number	Per cent
5.7 - 5.9	3	3.0	0	0
6.0 - 6.1	3	3.0	0	0
6.2 - 6.3	10	10.2	2	8.7
6.4 - 6.5	13	13.1	3	13.0
6.6 - 6.7	20	20.2	7	30.4
6.8 - 6.9	26	26.2	4	17.6
7.0 - 7.1	12	12.1	4	17.6
7.2 - 7.3	10	10.2	1	4.3
7.4 - 7.5	0	0	1	4.3
7.6 - 7.7	2	2.0	0	0
7.8 - 7.9	0	0	1	4.3
Total	99		23	

(7) Low vitamin A as compared to normal heifers.

Eight heifers were carried on a vitamin A deficient ration consisting of a concentrate mixture, beet pulp, weathered rye-grass straw of low carotene content and sufficient chopped, green, leafy alfalfa hay and vitamin A concentrate to meet the minimum maintenance requirements. In comparing the vaginal pH values of these animals with normal heifers receiving good hay and silage in winter and pasture in summer, a distinctly more alkaline range was found in the vitamin A deficient group. Sixty-one and two tenths per cent of the vaginal pH values of the normal heifers fell between 6.40 and 6.69 whereas 60.0 per cent of the vitamin A deficient group fell in the range of 6.70 to 6.99. The vitamin A deficient rations may have caused the higher vaginal pH values. The values

of these two groups of heifers are compared graphically in Figure 3.

2. Semen studies.

(1) Normal pH range of semen.

Hydrogen-ion concentration determinations were made on 343 samples of semen over a period of 14 months. Samples from 27 different bulls of three breeds, Jersey, Holstein and Guernsey were represented (Table 5). The highest pH reading recorded was 8.05 and the lowest determination was 6.15, the average of all determinations being 6.76. Of the 343 samples studied, 74.5 per cent had pH values falling between 6.50 and 6.90. These results compare very favorably with those of other workers (2, 11, 30, and 35). Of the 343 samples from these 26 bulls, 61 or 17.8 per cent had pH values greater than 6.90, and 70.5 per cent of the samples in this more alkaline pH range were from four bulls.

(2) Volume and pH.

In comparing initial pH and volume of each of the samples a general trend was observed for increasingly larger percentages of the samples to fall in the pH range of 6.50 and 6.80 as the volume of the ejaculates became larger. This is shown in Table 6. The pH range of 6.50 to 6.80 is used because it seems quite agreed in the review of literature that it is in this pH range that the

Figure 3 :-

NORMAL AND LOW VITAMIN A HIEFERS

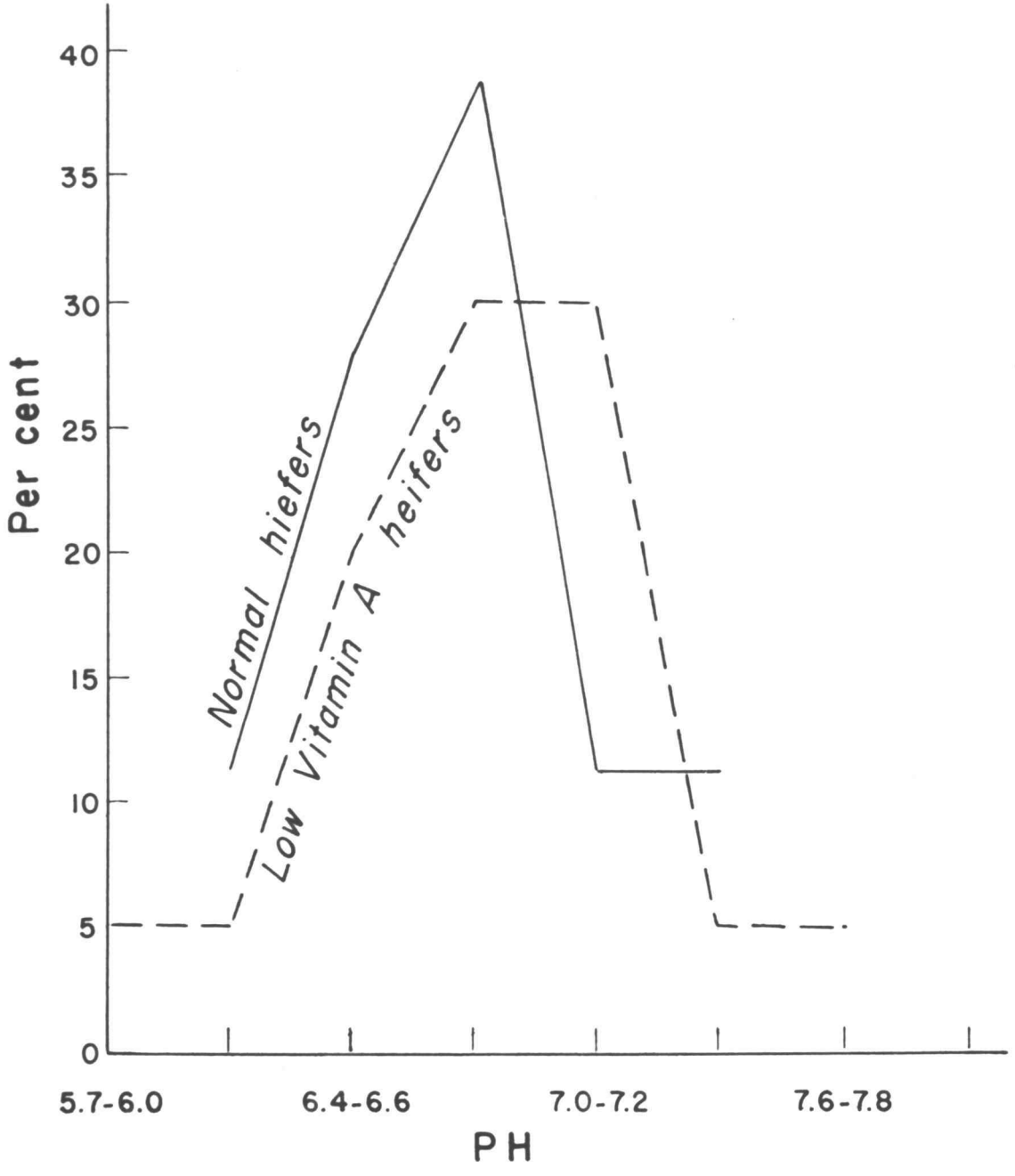


Table 5. PH OF BULL SEMEN

Breed and bull	No. of Samples	pH range	Average pH	Remarks
Jersey				
Butterking	26	6.30 - 7.05	6.66	OSC herd sire[1]
Foxie	56	6.50 - 8.05	6.91	OSC herd sire
J-17	3	6.63 - 6.87	6.76	ODBA sire[2]
J-28	4	6.60 - 6.98	6.73	ODBA sire
J-18	4	6.75 - 7.35	7.08	ODBA sire
J-22	3	6.25 - 6.60	6.45	ODBA sire
210 B	14	6.60 - 7.75	7.01	OSC exp. bull[3]
221 B	17	6.40 - 7.30	6.83	OSC exp. bull
218 B	11	6.70 - 7.55	7.00	OSC exp. bull
Breed average			6.82	
Holstein				
Pontiac	26	6.40 - 6.90	6.68	OSC herd sire
Cocky	17	6.30 - 7.00	6.52	OSC herd sire
Oley	5	6.65 - 7.45	6.88	OSC herd sire
Chuck	7	6.60 - 7.30	6.92	OSC herd sire
Master	10	6.50 - 6.75	6.60	OSC herd sire
H-13	3	6.65 - 6.80	6.76	ODBA sire
H-15	6	6.65 - 6.90	6.79	ODBA sire
H-2	4	6.25 - 6.75	6.48	ODBA sire
H-12	4	6.60 - 6.85	6.74	ODBA sire
521 B	10	6.40 - 6.93	6.65	OSC exp. bull
456 B	24	6.30 - 6.88	6.58	OSC exp. bull
522 B	20	6.50 - 7.20	6.87	OSC exp. bull
500 B	25	6.25 - 6.90	6.61	OSC exp. bull
496 B	15	6.70 - 7.15	6.93	OSC exp. bull
359 B	14	6.50 - 7.10	6.83	OSC exp. bull
Breed average			6.71	
Guernsey				
G-71	3	6.55 - 6.90	6.84	ODBA sire
G-72	9	6.40 - 6.68	6.58	ODBA sire
G-73	3	6.65 - 6.82	6.77	ODBA sire
Breed average			6.73	
Average all bulls			6.73	

1 Oregon State College herd sire.

2 Oregon Dairy Breeders Association sire.

3 Oregon State College experimental bull.

better quality semen is found.

Table 6. SEMEN VOLUME AND PH

No. of Samples	Volume c.c.	Average pH	Per cent samples from 6.50 to 6.80
23	1.00 - 1.99	6.91	26.0
68	2.00 - 2.99	6.78	54.0
56	3.00 - 3.99	6.79	52.0
28	4.00 - 4.99	6.77	60.5
24	5.00 - 5.99	6.72	75.0
31	6.00 - 6.99	6.67	71.0
18	7.00 - 7.99	6.65	78.0
18	8.00 - 8.99	6.65	83.5
8	9.00 - 9.99	6.73	75.0
11	10.00 - 10.99	6.63	91.0

In analyzing the data statistically by the method of analysis of variance according to Snedecore (37) significant differences exist between the volume of 1.00 to 1.99 c.c. and 2.00 to 2.99 c.c. and between 9.00 to 9.99 c.c. and 10.00 to 10.99 c.c. As shown in Table 6 as the volume increased from 1 to 10 c.c. per ejaculate, the average pH tended to become increasingly more acid, ranging from an average pH of 6.91 for the samples of 1.00 to 1.99 c.c. to 6.63 for the samples of 10.00 to 10.99 c.c.

(3) Motility and pH

In comparing motility with pH a trend similar to that of volume and pH was found. However, instead of being an inverse relationship as with volume the relationship is direct, the motility declining with the acidity.

The average pH for 131 samples rated as excellent was 6.68 with 68.5 per cent of these samples falling in the pH range of 6.50 to 6.80. The average for 115 samples rated as good plus was 6.75 with 61.0 per cent of the values being between 6.50 and 6.80. This same trend is followed by the subsequent grades with poor having an average pH of 6.91 and only 33.3 per cent of the values being between 6.50 and 6.80. This is shown in Table 7.

Table 7. SEMEN MOTILITY AND PH

No. of Samples	Motility	Average pH	Per cent samples from 6.50 to 6.80
131	Excellent	6.68	68.5
115	Good plus	6.75	61.0
29	Good	7.01	41.5
6	Fair	6.97	16.7
9	Poor	6.91	33.3

(4) Concentration and pH.

In comparing the concentration of semen with pH, the same direct relationship was found as existed with motility and pH. As the concentration increased, the acidity increased also. The samples of poorest concentration were neutral or slightly alkaline. The average pH of 67 samples of semen which were rated as excellent was 6.65 with 65.5 per cent of them in the 6.50 to 6.80 pH range, for 146 samples rated as good plus the average pH was 6.73 with 66.5 per cent of them between 6.50 and

6.80. Fifty-three samples rated as good averaged 6.81 with 41.5 per cent between 6.50 and 6.80. Thirteen samples rated as fair averaged 6.94 with 16.7 per cent between 6.50 and 6.80 and four samples rated as poor had an average pH of 7.10, 33.3 per cent of which were between pH 6.50 and 6.80 (Table 8).

Table 8. SEMEN CONCENTRATION AND PH

No. of Samples	Concentration	Average pH	Per cent samples from 6.50 to 6.80
67	Excellent	6.65	65.5
146	Good plus	6.73	66.5
53	Good	6.81	41.5
13	Fair	6.94	16.7
4	Poor	7.10	33.3

When analyzed statistically, a significant difference in pH was found between the samples rated excellent and good plus, excellent and good, and good plus and good.

A complete ejaculate is composed of definite proportionate amounts of accessory gland secretions and the acid epididymal fraction with the sperm. If, due to over abundant accessory gland secretion or an incomplete ejaculation, the proper proportionate amounts are altered so as to reduce the acid epididymal fraction, the acidity of the whole ejaculate is reduced and thus one might expect to find a more alkaline condition in those samples of low concentration.

(5) Monthly pH variation.

In comparing the average pH of the semen samples collected during the different months of the year, considerable variation is found. The lowest pH values occurred in the months of December and January with averages of 6.70 and 6.48 respectively. The high for the year was reached in April and May with values of 6.82 and 7.05 (Table 9).

Table 9. MONTH AND PH

Month	No. of Samples	Average pH	Per cent between 6.50 and 6.80
Jan.	28	6.48	93.0
Feb.	19	6.76	68.5
April	5	6.82	60.0
May	8	7.05	25.0
June	49	6.80	51.0
July	70	6.76	57.0
Aug.	58	6.77	55.0
Sept.	36	6.80	50.0
Oct.	4	7.00	50.0
Dec.	20	6.70	90.0
Dec., Jan., Feb., Winter period	67	6.61	83.8
June, July, Aug., Summer period	177	6.70	54.3

When analyzing the data statistically by the method of analysis of variance significant differences can be shown between the pH values of the semen samples collected in January and those collected in April, also between February and April, May and June, May and July,

December and January, December and February, December and May, December and June, December and July, and December and September. From this one might conclude that a monthly and possibly a seasonal variation in pH of bovine semen does exist.

In comparing the three mid-winter months, December, January, and February as a group with the three mid-summer months, June, July and August, a distinct difference is shown both in average pH and in per cent of samples between pH 6.50 and 6.80 (Table 9). The mid-winter group of 67 samples had an average pH of 6.61 with 83.8 per cent of the samples having pH values of between 6.50 and 6.80. The mid-summer group which consisted of 177 samples had an average pH of 6.70 with only 54.3 per cent of them falling in the 6.50 to 6.80 range.

(6) Comparison of first and second ejaculates with pH.

The average pH for 155 first ejaculates of semen was 6.60 as compared to an average pH of 6.75 for 101 second ejaculate determinations. This is not as great a difference as reported by Davis and Williams (11), however, it does indicate that first ejaculates are more acid than are second ejaculates. The distribution of the samples in the various pH ranges, as given in Table 10, points to this conclusion.

Table 10. PH OF FIRST AND SECOND EJACULATES

pH range	First ejaculate		Second ejaculate	
	No. of Samples	Per cent	No. of Samples	Per cent
6.1 - 6.39	5	3.2	1	1.0
6.4 - 6.59	34	22.0	8	7.9
6.6 - 6.79	68	44.0	40	39.5
6.8 - 6.99	34	22.0	31	30.6
7.0 - 7.19	10	6.5	13	12.9
7.2 - 7.39	2	1.3	6	5.9
7.4 - 7.59	1	0.6	2	1.9
7.6 - 7.79	1	0.6	0	0

(7) Age of bull and semen pH.

The pH values for 150 semen samples representing ten bulls under two years of age were 6.79, as compared to 6.70 for 131 samples representing 20 bulls between two and ten years of age. The concentration is also lower for the bulls under two years old. It has been shown before that the trend is for pH to increase as concentration decreases. Therefore, the lower pH for the older bulls might be explained in this way. It may be seen in Table 11 that for the bulls under two years only 51.7 per cent of the samples are below pH 6.70 whereas for the bulls two years and over 69.4 per cent of the samples fall below 6.70.

3. Change in semen characteristics following incubation.

(1) Motility and pH change.

The pH of 59 samples of semen with a motility rating of excellent varied from 6.25 to 6.92, the average

Table 11. AGE OF BULL AND PH OF SEMEN

pH range	Bulls under two years of age		Bulls two years of age and over	
	No. of Samples	Per cent	No. of Samples	Per cent
6.1 - 6.3	3	2.3	3	2.3
6.4 - 6.5	21	14.0	23	17.6
6.6 - 6.7	53	35.4	65	49.5
6.8 - 6.9	42	28.0	28	21.4
7.0 - 7.1	22	14.7	7	5.3
7.2 - 7.3	5	3.4	4	3.1
7.4 - 7.5	3	2.0	1	0.7
7.6 - 7.7	1	0.7	0	0
Total	150		131	

being 6.65 (Table 12). On incubation for one hour both pH and motility decreased from the initial value in all but three cases in which the motility did not change. In 53 samples a further but not quite as large decrease occurred in both pH and motility during the second hour of incubation. In the case of three samples, a slight increase in pH occurred during the second hour and in three other samples in this group the pH remained the same as at the end of the first hour.

A second group of 23 samples rated as good plus varied from 6.52 to 7.15 with an average pH of 6.76. They followed the same trend as did the first group upon incubation. However, the decreases in motility and pH at the end of incubation for two hours were not quite as large as in the first group.

Table 12. MOTILITY AND PH OF UNDILUTED SEMEN HELD AT 38° C. FOR TWO HOURS

Rating	No. Samples	Initial		After one hour		Decrease		After two hours		Decrease		Total decrease	
		Motility	pH	Motility	pH	Motility	pH	Motility	pH	Motility	pH	Motility	pH
Excellent	59	9.20	6.65	5.70	6.00	3.50	0.65	3.06	5.80	2.64	0.20	6.56	0.85
Good plus	23	7.65	6.76	4.44	6.25	3.21	.51	1.96	6.05	2.48	0.20	5.69	0.71
Good and fair	23	4.20	6.80	1.44	6.25	2.76	.55	1.00	6.10	0.44	0.15	3.20	0.70

A group of 23 samples having ratings of good and fair varied from 6.50 to 7.35 with an average pH of 6.80. The decrease in pH value was very similar to that of samples rated good plus.

The rate at which the pH fell varied from sample to sample. It was the most rapid during the first hour. The decrease in pH at the end of two hours was 0.85 pH units for those samples with motility ratings of excellent. For the groups of samples with motility ratings of good plus and good and fair the pH decrease was 0.71 and 0.70 pH units respectively. The initial pH values were the lowest for the samples with the best motility and highest for the poorer samples. The pH change tended to be greatest for the samples having the best motility.

(2) Concentration and pH change.

In comparing pH change with concentration of 100 samples of semen after incubation the same relationship existed as was found with motility. The higher the the concentration of the semen the greater the decrease in pH (Table 13). The average initial pH of 44 samples having a concentration rating of excellent was 6.70. The decrease in pH at the end of the first hour of incubation was .60 pH units, the total decrease following incubation for two hours being .80 pH units. The average initial pH

Table 13. CONCENTRATION AND PH OF UNDILUTED SEMEN INCUBATED AT 38° C.

Rating	No. of samples	Initial pH	pH after one hour	Decrease	pH after two hours	Decrease	Total decrease
Excellent	44	6.70	6.10	0.60	5.90	0.30	0.80
Good plus	48	6.74	6.20	0.54	6.00	0.20	0.74
Good and fair	8	6.70	6.35	0.35	6.10	0.25	0.60

for 48 samples rated as good plus was 6.74. A decrease of 0.54 pH units followed the first hour of incubation and for the two hour period the average decrease was 0.74. The initial average pH of eight samples rated as good and fair was 6.70. The decrease at the end of the first hour was 0.35 and the decrease for two hours was 0.60. The relative decrease was considerably greater for the second hour of incubation with the samples of lower quality than for the first hour for these same samples or for the second hour of the samples of better quality.

(3) pH change and conception rate.

The pH determinations on semen used to inseminate cows for which conception results were available are presented in Table 14.

On comparing the final pH and the conception rate, a definite inverse relation exists. The seven samples having the lowest final pH ranging from 5.60 to 5.90 and averaging 5.80 had a 74.2 per cent non-return rate based upon first services only. The seven samples having the highest final pH which ranged from 5.95 to 6.10 and averaged 6.00 had a 68.2 per cent non-return rate. The seven samples having the greatest decrease in pH following the two hour incubation have a slightly higher per cent non-return rate than the seven samples showing the least drop in pH. This is shown in Table 15. It may also

Table 14. COMPARISONS OF INITIAL AND OF FINAL PH AND CONCEPTIONS

Bull	Initial pH	Per cent nonreturn	Bull	Final pH	Per cent nonreturn
			Highest group		
G-70	6.87	63.6	G-74	6.10	66.7
J-17	6.87	73.8	G-72	6.10	60.5
H-13	6.80	66.7	G-72	6.08	78.4
H-15	6.70	66.7	G-72	6.00	50.0
G-74	6.70	66.7	J-17	6.00	73.8
H-13	6.70	84.0	H-15	5.95	66.7
G-72	6.68	78.4	J-28	5.95	81.5
Average	6.77	71.5		6.00	68.2
			Lowest group		
G-72	6.65	50.0	H-2	5.90	82.2
H-15	6.65	67.7	H-2	5.90	71.0
J-28	6.60	81.5	H-13	5.90	66.7
G-71	6.55	84.5	G-70	5.80	63.5
H-2	6.48	82.2	H-13	5.75	84.0
H-2	6.45	71.0	H-15	5.70	67.7
G-72	6.40	61.0	G-71	5.60	84.5
Average	6.55	71.3		5.80	74.2

be noted in Table 15 that in every case where the same bull is represented by two or more samples all of the samples based on decrease in pH following incubation are found in the same half, either upper seven or lower seven, of the determinations. In no case is the variation between samples of the same bull great enough to have him represented in both the lower and upper half of the group.

Table 15. PH CHANGE AND CONCEPTION RATE

Bull	Number of services	Initial pH	Final pH	Decrease in pH two hours	Per cent nonreturn
G-70	30	6.87	5.80	1.07	63.5
G-71	26	6.55	5.60	.95	84.5
H-13	25	6.70	5.75	.95	84.0
H-15	31	6.65	5.70	.95	67.7
H-13	27	6.80	5.90	.90	66.7
J-17	42	6.87	6.00	.87	73.8
H-15	24	6.70	5.95	.75	66.7
Average group I			5.82		72.5
J-28	43	6.60	5.95	.65	81.5
G-72	38	6.65	6.00	.65	50.0
G-72	37	6.68	6.08	.60	78.4
G-74	18	6.70	6.10	.60	66.7
H-2	28	6.48	5.90	.58	82.2
H-2	24	6.45	5.90	.55	71.0
G-72	43	6.40	6.10	.30	60.5
Average group II			5.90		70.0
Average group I and II			6.00		71.2

SUMMARY

It was found, in making 145 *in vivo* vaginal pH determinations representing 66 different animals, that the largest per cent of the determinations fell in the pH range of 6.70 to 6.99.

A comparison of 72 determinations of animals receiving four or more services per conception which were classed as difficult breeders with 28 determinations of animals requiring three services or less per conception and classed as normal was made. There was a tendency for the pH values of the difficult breeding group to be slightly more alkaline than the normals, but the difference was not significant.

It was found in comparing the pH values of pregnant and open cows that the values for the pregnant cows were slightly more alkaline.

A group of eight heifers carried on a vitamin A deficient diet showed a more alkaline condition of the genital tract than did the animals on a normal ration.

In determining the pH of fresh undiluted semen from 27 different bulls over a period of 14 months, a range from 6.15 to 8.05 with an average of 6.73 was found.

Upon comparing initial pH with volume of ejaculate a general trend existed for increasingly greater percentages of the determinations to fall in the pH range of

6.50 to 6.80, which appears to be the range of most fertile semen, as the volume of the ejaculates increased.

When comparing motility with pH a direct relationship was found to exist in that as the motility declined so also did the acidity.

A comparison of concentration of semen with pH showed the same direct relationship. Acidity decreased with lowered concentration.

A variation in pH of samples for the various months was shown. The lowest pH values occurred in December and January and the highest in April and May. Also a distinct seasonal difference existed between the winter and summer months.

The average pH for first ejaculates was found to be slightly more acid than for second ejaculates.

Age of bull appears to have some effect on pH of semen. The samples from bulls under two years of age was slightly more alkaline than for the bulls over two years old.

Incubation of fresh, undiluted semen at 38° C. resulted in increased acidity of the sample. The increase was found to be greatest for those samples having the best motility. This same relationship existed when comparing pH change with concentration of the ejaculate. On comparing change in pH following incubation with conception

rate a definite inverse relationship was found. The group composed of 50 per cent of the samples, having the lowest final pH, had the highest conception rate.

CONCLUSIONS

The data seem to indicate that conception rate is higher for cows having slightly more than average acid condition of the genital tract.

Semen samples falling in the more acid range appear to be of higher fertility.

A low pH and a large decrease in pH following incubation for two hours at 38° C. indicates measures of fertility of bull semen.

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