

AN ABSTRACT OF THE THESIS OF

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Title-----In Vivo Determination of the Hydrogen Ion
Concentration of the Vaginas of Domestic Animals-----

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This study includes vaginal pH determinations made on several species. Special statistical emphasis is placed on the vaginal pH determinations made on 400 cows from 15 herds in western Oregon. Eight of these herds were selected for study because they were having considerable breeding difficulty. Vaginal pH determinations were made during the breeding season on 53 cows in these herds.

The determinations were made in vivo, using a Beckman pH meter, with a glass electrode assembly, constructed especially for this purpose. The customary potassium chloride calomel electrode in this assembly is replaced by a silver-silver chloride electrode. The electrode is connected to the pH meter by a ten-foot lead.

It was felt that in vivo pH determinations would be more representative of actual vaginal pH values, since it is a known fact that many body fluids undergo rather rapid changes in pH after being removed from the body and exposed to air.

In this work special emphasis is placed on the possibility of any correlation between vaginal pH and breeding efficiency.

Vaginal pH values obtained by the in vivo method are somewhat lower than those reported from determinations made on vaginal secretions or on washings removed from the animals.

In cows the lowest value obtained is pH 5.52, and the highest pH 8.00. The highest percentage of pH values (44.75%) varies between 6.51 and 7.00.

The limited number of pH determinations made on other species (horse, sheep, swine, goat) indicates that species variations might exist. The values obtained from 10 sheep and two swine are somewhat higher than comparable values obtained from other species.

Although eight of the cow herds included in the study were having considerable breeding difficulty, no correlation is noted between vaginal pH and breeding efficiency. Neither is there any apparent correlation between pathological changes in the vaginal mucosa and pH.

A single douching experiment was performed, using one-half per cent lactic acid on one animal and one-half per cent sodium bicarbonate on another animal. Hydrogen ion concentration changes were rather marked, and persisted until approximately four hours after douching. The vaginal pH of the cow douched with one-half per cent lactic acid showed a

pH decrease from 6.94 to 2.83. In four and one-half hours the vaginal pH was 7.32 or slightly higher than the pre-douching level.

IN VIVO DETERMINATION OF THE HYDROGEN ION
CONCENTRATION OF THE VAGINAS
OF DOMESTIC ANIMALS

by

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IN VIVO DETERMINATION OF THE HYDROGEN ION CONCENTRATION OF THE VAGINAS OF DOMESTIC ANIMALS

During the past decade a number of persons working in the field of reproductive physiology have centered their attentions on the possibility of normal and abnormal variations in the hydrogen ion concentration of the vaginas of females. Since it has been shown that such variations do exist in certain animals, recent work has been concerned with causes, and the possible effect on breeding efficiency.

Recently these studies have been given impetus by the widespread interest shown in artificial insemination, and by theories advanced on the effect of vaginal hydrogen ion concentration on sex determination.

Studies of the hydrogen ion concentration of the vaginal secretions of cows have been made by McNutt, Schwarte and Eveleth⁽⁶⁾, and in vivo studies of women have been made by Trussel⁽⁸⁾. In previous work, the numbers of individuals studied were rather limited.

Most of the hydrogen ion concentration determinations have been made on vaginal secretions or on washings obtained by washing out the vaginal secretions with physiological saline and thoroughly mixing.

Since it has been shown⁽³⁾ that some body fluids undergo rather rapid changes in hydrogen ion concentration when exposed to air, it was felt that in vivo determinations

might be somewhat more representative of actual vaginal pH value. (In this work hydrogen ion concentration will be expressed in the conventional manner, as pH.)

Studies of a large number of individuals under actual field conditions seemed highly desirable. It is felt that sufficient numbers of cows have been studied to be representative of in vivo vaginal hydrogen ion concentration in all phases of the estrus cycle and in all stages of gestation. Comparative vaginal pH values in animals of high and low breeding efficiency were studied. A limited number of determinations have been made on other domestic animals.

Review of Literature

Data presented by McNutt, Schwarte, and Eveleth⁽⁶⁾ and based on examinations of the vaginal secretions of eight virgin heifers and three cows, varied between pH of 5.10 and 8.80. The pH determinations were made at frequent intervals over varying periods, generally covering one or more complete estrus cycles. Cows in late pregnancy showed a variation between pH 7.00 and 8.80. After calving, the range was similar, but averaged slightly lower, varying between 6.00 and 7.00. Heifers varied between 5.10 and 8.80. Unspayed heifers varied between 6.20 and 7.30 and spayed heifers between 6.25 and 7.10. Except for one case,

all determinations were above neutral at the time of heat. The general trend of these results suggests that pH values of the cow's vagina is of small significance for diagnostic purposes in breeding diseases.

Beilly⁽¹⁾ has shown that there is a changing pH in vaginal fluid in rats that is cyclic in occurrence. The acid phase is associated with "epithelization" and estrus; the alkaline phase with the leucocytic or diestrus stage. When leucocytes predominate, the pH of vaginal fluid changes toward alkalinity. It was observed in this work, too, that the pH of vaginal fluid varies little in the castrate rat.

In previous work⁽²⁾ Beilly had noted that the vaginal pH of castrate humans was alkaline. When estrogens were administered to these individuals, the vagina changed from alkaline to the acid phase until normal pH was attained. Pinpoint ulcerations in the vestibular and vaginal mucosa disappeared with the treatment. A definite correlation was noted between pH of secretions and cytological changes in the vaginal mucosa. The acid phase is associated with the high follicular phase.

There has been some disagreement about the changes in vaginal pH associated with estrogen stimulation. Dow and Zuckerman⁽⁴⁾ observed the very important fact that the same degree of estrogenic stimulation is not necessarily associated with the same pH in different animals, or in the same

animal at different times. Their work indicates that the dose level of estrogens varies in different individuals of the same species and in the same individual at different times. It was shown also that progesterone injections following a period of daily injections of estrogenic hormone caused a rise in vaginal pH, and that uterine bleeding is generally preceded by a rise in vaginal pH.

Oberst and Plass⁽⁷⁾, using a quinhydrone micro-electrode on samples obtained from women (177 determinations), concluded that (1) the vaginal discharge pH varies directly with the character of the vaginal flora, higher acidities being associated with a preponderance of Doderlein's bacilli, which were normally present in pure cultures, but were absent in certain infections (gonorrhea); (2) the vaginal discharge is normally quite acid (pH 4.00 to 4.50) during the intermenstrual period, but approaches or exceeds neutrality during the early days of menstrual bleeding; (3) during gestation the acidity is somewhat less than in non-pregnancy, irrespective of the bacterial character of the discharge; (4) the pH of the posterior part of the vagina is slightly lower than the anterior portion; (5) dilution of vaginal discharge with neutral water tends to raise the pH, although the acidity of the vaginal washings may be higher than that of the undiluted discharge.

Trussell⁽⁸⁾ made the first in vivo vaginal pH determination. His work is reviewed here in some detail, since the technique is very similar to the one used in obtaining the data reported in this paper.

Trussell used a Cameron pH meter (containing an amplifying tube and carrying a saturated calomel electrode and a glass electrode), calibrated directly in terms of pH. A ten-foot connecting cable between the potentiometer and the amplifying unit and electrodes gave the unit flexibility.

With the patients in the lithotomy positions, the glass electrode was inserted into the vagina to the desired depth, and the circuit completed by placing in the vagina beside the electrode a sturdy, blunt, pointed capillary tube which was connected with the potassium chloride reservoir of the calomel electrode by a rubber tube filled with saturated potassium-chloride. The difference in potential between the two electrodes depends upon the pH of the vaginal discharge and was indicated as such on the potentiometer.

The glass electrodes were cleansed between tests with soap and water and alcohol. When not in use they were kept in water. The potassium-chloride junctions are not so easily cleansed. Several were kept available. They were immersed (when cleaned) for ten minutes in 2% phenol, rinsed with distilled water, and refilled with saturated KCl.

The authors experienced difficulty early in the work with (1) faulty glass electrodes due to "bubble etching," (2) contact of the glass electrode with the cervix, exposing the electrode to an alkaline cervix and an acid vaginal environment, making readings impossible (By pulling the electrode away from the cervix, this difficulty was obviated.), (3) failure to complete the circuit so that no reading could be obtained. Rotation of the glass electrode and of the KCl junction tube, and occasionally flushing a small amount of the KCl solution through the tube usually restored normal operating conditions promptly.

Readings were taken at different levels in the vagina and studies were made of the bacterial flora. Two hundred ante-partum women were included in the study.

This work indicated that in late pregnancy the acidity is highest in the middle portion of the vagina, with the lower vagina being less acid, and the cervix and upper vaginal fornices having an alkaline or only slightly acid reaction.

There seems to be some correlation between the acidity variations in the middle vagina and the type of vaginal flora, especially when the Doderlein's bacilli are present. All of the variations in vaginal flora cannot be explained by alterations in vaginal acidity.

Methods and Procedure

This report includes vaginal pH determinations made on 400 cows from 15 dairy herds in western Oregon. Eight of the herds were selected for study because they were having considerable breeding difficulty. Vaginal pH determinations were made during the breeding season on 53 cows in these herds.

A Beckman pH meter was used in this work, using a glass electrode assembly constructed especially for this purpose. The customary potassium chloride calomel electrode in this assembly is replaced by a silver-silver chloride electrode. This electrode is located on the body of the glass electrode some four inches from the ground portion and connects through the glass to the lead wire provided with a phone tip. The electrode and extension jacket measures $23\frac{1}{2}$ inches, and is connected to the pH meter by a ten-foot lead.

The electrode was inserted into the vagina until it was felt that the tip was near the anterior part of the vagina or near the external os of the cervix. Three readings were taken within $1\frac{1}{2}$ minutes and the average taken as the vaginal pH value at that time. Occasionally there was considerable difference in the readings due to straining and body movements of nervous individuals. Some of these irregular readings may have been due to the fact that the electrode was in contact with the cervix and the anterior

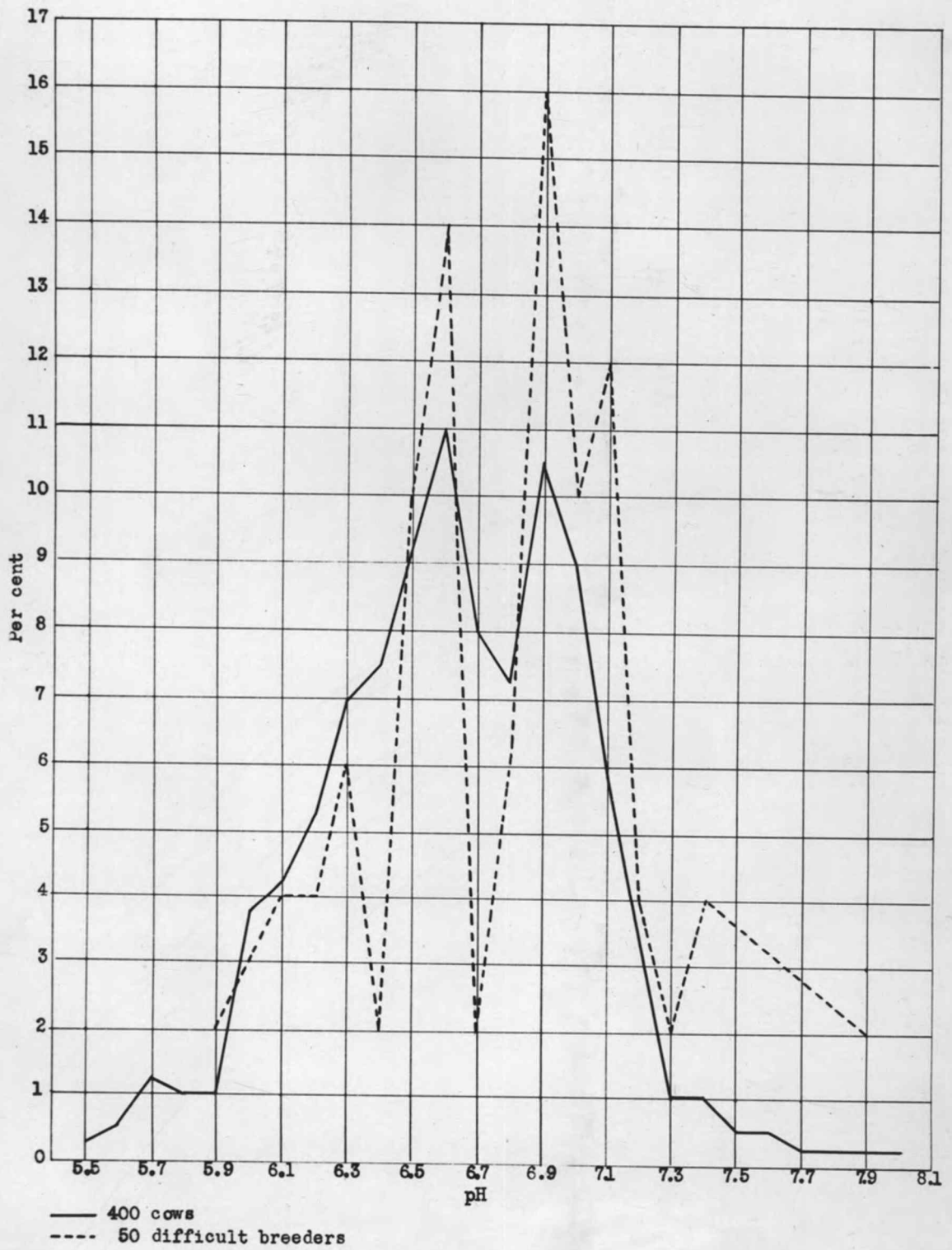
part of the vagina, giving an alkaline and acid contact at the same time, making correct readings impossible. These readings are not included in this report. Breeding histories were obtained on each individual as completely as possible.

In this study the highest pH reading obtained was 8.00 and the lowest 5.52. Four and seventy-five hundredths per cent varied between 5.52 and 6.00, 29.00 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 7.05, and 1.75 per cent between 7.51 and 8.00.

Vaginal pH values of 50 cows classed as difficult breeders varied from 5.99 to 7.46. All cows having four or more services, whether pregnant or not, were classed as difficult breeders. The only pH reading below 6.00 was 5.99. This cow conceived on the fourth service. One cow with a vaginal pH of 6.99 was still non-pregnant after 18 services. Twenty-two of the difficult breeders were known to be non-pregnant. No correlation was noted between breeding efficiency and vaginal pH. The pH recordings of the difficult breeding cows is compared graphically with recordings of the entire group of cows in Figure 1.

It will be noted from the frequency distribution curve in Figure 1 that there appears to be a tendency for the pH values to group themselves around pH 6.60 and 6.90.

Figure 1



Whether this distribution represents a significant physiological difference has not been determined. Statistical calculations indicate that such variations may well be due to chance.

The normal distribution corresponding to the group of 400 pH values is compared with the actual distribution curve in Figure 2.

The vaginal pH values varied as much for pregnant as for non-pregnant cows. Relatively accurate breeding histories were obtainable on 335 cows. The average pH value of the 196 cows that were pregnant was 6.58, and the average pH of the 139 that were non-pregnant was 6.68.

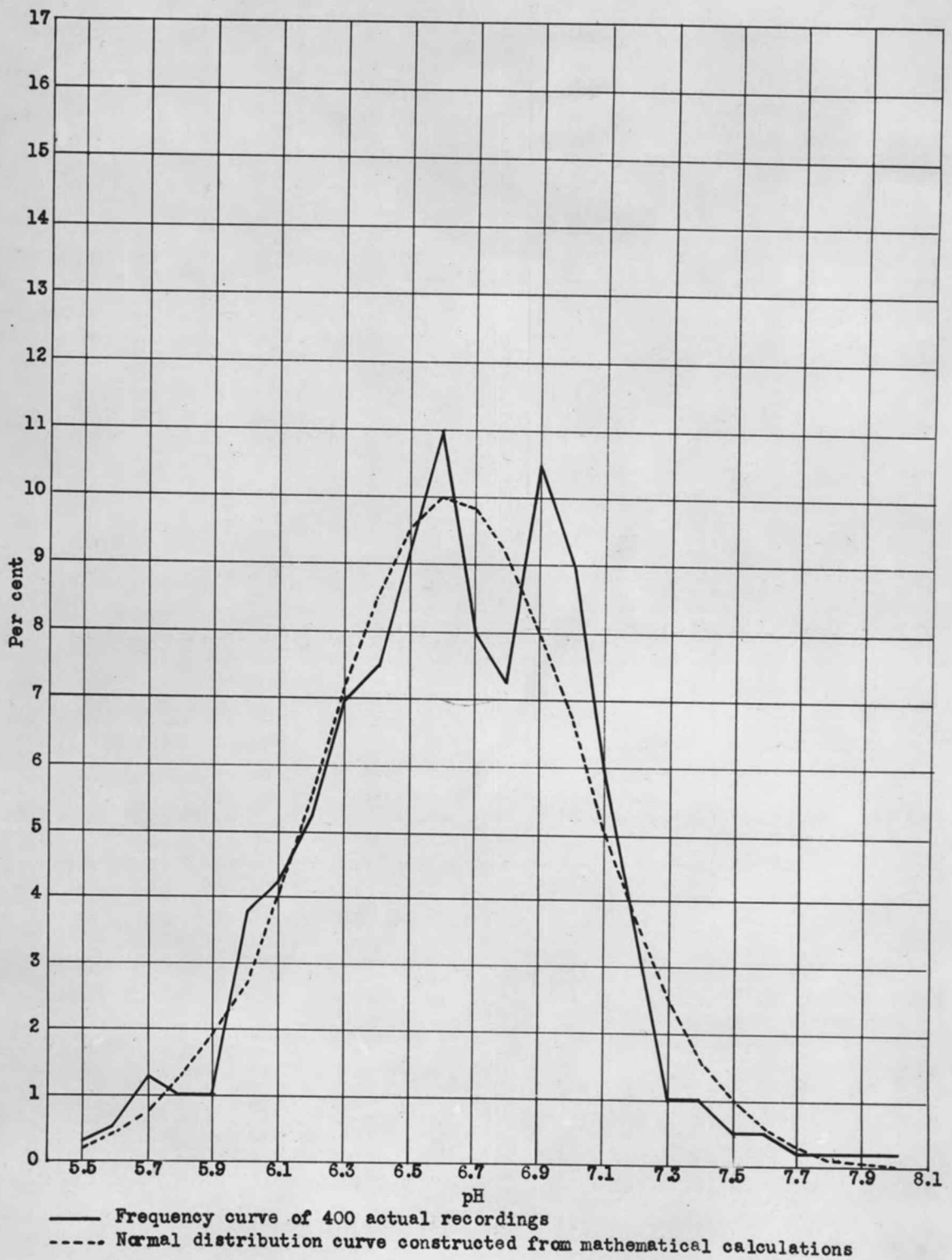
Six cows were known to be in heat at the time the readings were made. These values varied from 6.72 to 7.08, five of them being below 7.00.

The average vaginal pH of 13 virgin heifers was 6.27 with values varying from 5.65 to 7.05. Seventeen pregnant heifers in the same herd had an average pH of 6.36 with values varying from 6.00 to 7.15.

Ninety-one cows showed evidence of having granular vulvo-vaginitis. The average pH of this group of cows was 6.69 with values varying between 7.75 and 5.71.

Thirty vaginal pH determinations were made over a period of 35 days. Four of the five cows used in this study were pregnant, cow No. 2 being non-pregnant. These

Figure 2



recordings are shown graphically in Figure 3, and indicate the day-to-day variation that may be expected in the same animal.

Vaginal pH determinations were made on ten ewes, three mares and two sows, and one goat.

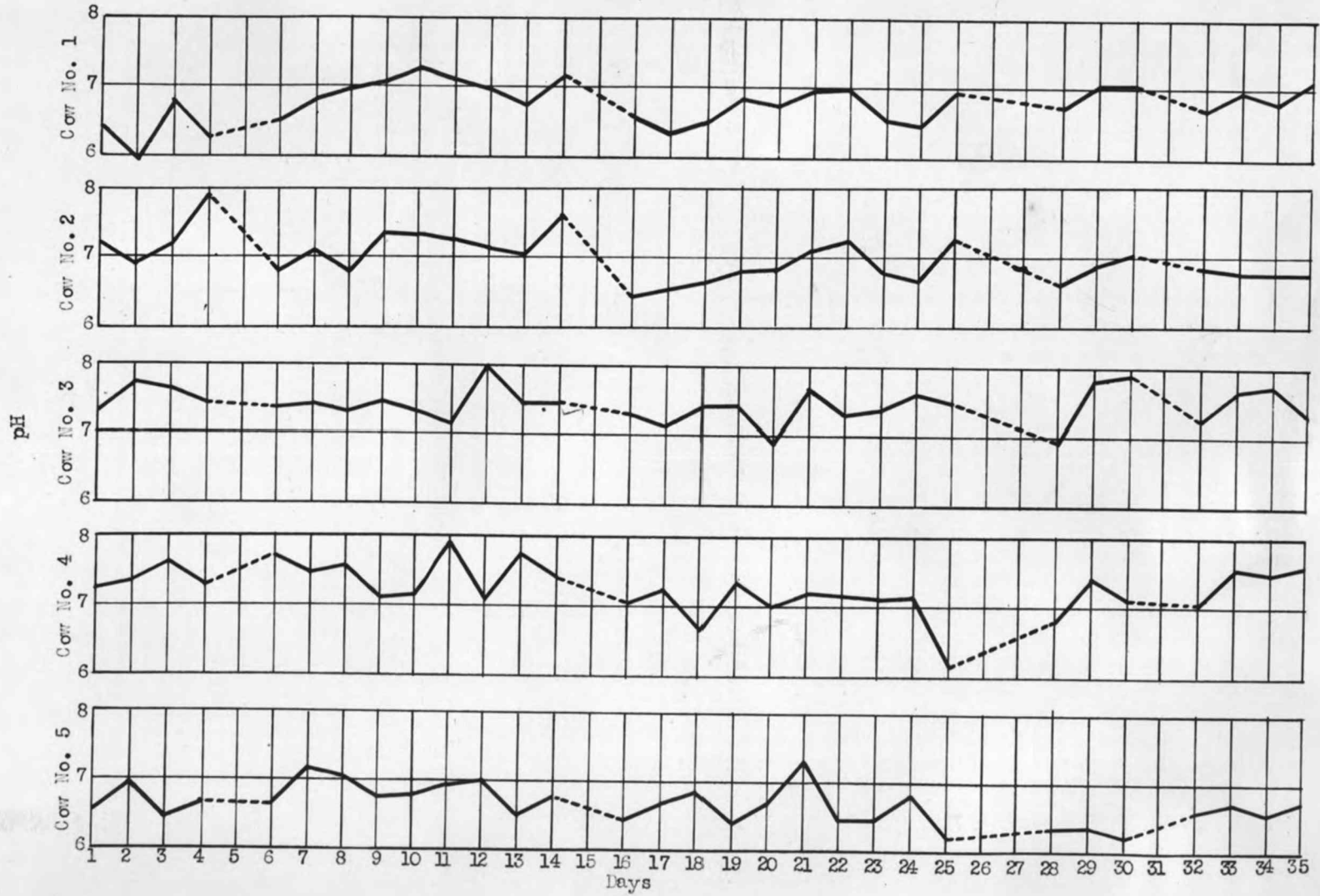
Eight of the ewes had recently lambed, one was in the late stages of pregnancy, and one was non-pregnant. The pH determinations varied between 6.56 and 7.70. Only one ewe was below pH 7.00. This animal was in the recent post partum state. The one determination made on the non-pregnant goat was pH 6.88.

The three determinations made on mares were 6.96, 6.87 and 6.82. All were non-pregnant, the first mare being in the recent post partum state.

The determinations made on two non-pregnant sows were 7.12 and 7.01.

During the past few years some experimental work done in private laboratories indicates that vaginal pH may have an influence on sex determination and that a preponderance of male offspring may be expected when the females are douched with mild alkaline solutions one-half to one hour before coitus. A preponderance of female offspring may be expected when the female is douched with mild acid solutions (lactic acid) one-half to one hour before coitus. These "theories" have received much publicity, but have not been

Figure 3



confirmed by careful workers.

The apparatus used in this work made possible the determination of actual in vivo pH changes that could be expected from such douche treatments.

Two cows were used in this experiment. Determinations were made before douching and at frequent intervals afterward. One cow was douched with one-half per cent sodium bicarbonate (pH 8.20), and the other with one-half per cent lactic acid (pH 2.54).

The following table shows the results obtained.

Figure 4

Cow	Douche used and pH	Vaginal pH								
		Pre- douche	After douching							
			5 min.	30 min.	50 min.	60 min.	70 min.	90 min.	4 hrs.	4½ hrs.
1	½% NaHCO ₃ pH 8.20	6.74	7.84	8.02	7.94	7.88		6.90		7.49
2	½% C ₃ H ₅ O ₃ pH 2.54	6.94	2.83	4.08			4.98		7.32	

Discussion

The results of this study seem to indicate that very low or very high vaginal hydrogen ion concentrations are rather unusual in domestic animals. There is some indication that certain species variations do exist, although the numbers of other species studied in this work are too

small for such evidence to be conclusive.

The results in this work are slightly lower than those reported by McNutt, Schwarte and Eveleth. It is possible that the slight difference may be due to differences in technique, or it may be due to the fact that body fluids and secretions sometimes undergo rapid changes in pH when exposed to air.

In the majority of animals the readings were extremely constant, but in some individuals there was some variation in the readings even in quiet animals. This may have been due to placement of the electrode too near the cervix as previously mentioned, or to the lack of secretions in the vagina.

The variations induced by douching are no doubt quite variable in different individuals, both as to amount and duration of the variations. Assuming that such variations do exist, then vastly different results would be obtained with the same strength douching solutions.

It is a known fact that spermatozoa are extremely sensitive to rapid changes in the pH of their environment. Sperm are also sensitive to hypertonic solutions. A common method of counting spermatozoa consists in using a regular hemacytometer and red cell diluting pipette. Two or three per cent salt solution used as the diluting fluid will inactivate and apparently kill sperm in less than a minute.

Conclusions

1. In vivo vaginal pH studies were made in 15 herds including 400 cows, using a glass electrode designed especially for this purpose.

2. The highest recording was a pH of 8.00 and the lowest 5.52.

3. The determinations were distributed as follows:

4.75 per cent varied between 5.52 and 6.00,
29.00 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.

4. Although eight of the herds studied were having considerable breeding trouble at the time the study was made, no correlation was noted between vaginal pH values and the extent of the breeding trouble, further substantiating previous work to which reference has been made.

5. In vivo vaginal pH determinations were made on ten ewes, three mares two sows and one goat. The sheep and sow recordings were slightly higher than those obtained in other domestic animals.

6. Marked variations in vaginal pH were obtained by douching two cows, one with one-half per cent sodium bicarbonate and the other with one-half per cent lactic acid. The pH of the vaginas returned to approximately the pre-douching level in four and one-half hours.

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