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TABLE OF CONTENTS

1. Introduction.

2. Physiology of Ascorbic Acid.

3. Experimental Data
   (a) Injection of the Dairy Bull
   (b) Injection of the Dairy Cow

4. Discussion.

5. Summary and Conclusion.

6. References.
Introduction:

Sterility in domestic animals has long presented a major farm problem. Due to the important role played by the dairy cow in her production of food, sterility in dairy animals has become more important than the same factor in other domestic animals. Coupled with the increasing importance of dairy animals is the modern breeding program, designed to meet the ever increasing demand for higher milk production. Naturally there are limits to the production of milk from normal healthy animals, and as these limits are attained and passed, the natural elements in the animal’s body become depleted. This is being demonstrated by the increasing prevalence of parturient paresis, acetonemia, and other metabolic disturbances associated with high production. In the last few years there has been an ever increasing interest shown in the sterility problem— with a view to its solution. In fact it has been only during the last two decades that the treatment of sterility has been attempted.

All sterility cases may be placed in one of the two following categories: (a) Where there is a definite pathological condition which hinders the functions of reproduction. (b) Where no definite pathological condition can be found. The former responds to treatment that corrects the pathological condition, but the apparent cause for the sterility in non pathological cases has proven most baffling.

The latter type of sterility remained a mystery until Paul H. Phillips and associates at the Agricultural Experimental Station of the University of Wisconsin, made some interesting discoveries. While carrying on the routine of analyzing animal tissue they came upon certain facts that demonstrated the relationship of certain glands of the body with Vitamin C. As early as 1933 they noticed that the anterior lobe of the pituitary gland was remarkably high in ascorbic acid. That in itself was not enlightening, but it raised the question “Why?” In 1938 the trail became warmer when analysis of blood plasma indicated that cattle on certain types of rations may have a Vitamin C deficiency.

It was about this time that they found that there is a bond between Vitamin A and
Vitamin C. Animals fed on rations short in Vitamin A showed a secondary effect in the form of a Vitamin C deficiency. The exact relationship of the two vitamins is a mystery, but there is a connection beyond a doubt. Phillips and associates first gathered observational evidence and later carried on experiments. An eye disease known as papillary edema, caused by Vitamin A deficiency, was completely prevented by feeding carotin and injecting ascorbic acid. Using carotin alone, feeding it in Vegetable Oils did not prevent the trouble in all cases, nor did ascorbic acid injections alone prevent it. However one calf which already showed Vitamin A deficiency responded markedly to injections of ascorbic acid without feeding carotin.

Physiology of Ascorbic Acid

Until recently ascorbic acid was thought to be valuable only in prevention of scurvy in man, monkey and the guinea pig. Vitamin C is primarily concerned with the production and maintenance of normal material between the body cells. This intercellular material is normally liquid, and the supposition is that it changes to a solid or jelly-like state when Vitamin C is not supplied in the food. These changes are marked in the intercellular structure of the teeth and bones. In Vitamin C deficiency the cells that produce intercellular substances undergo striking changes. Tiny capillaries become weakened and tiny hemorrhages occur throughout the body. Since the war Vitamin C has been used to assist in the proper healing of wounds.

Bleeding, loose teeth, swollen joints and porous fragile bones have been characteristic symptoms of scurvy. Cattle have never been known to show these symptoms of scurvy. This is due primarily to the fact that the dairy animal can synthesize their own Vitamin C, and thus maintain some of the needs of their body. Wallis (12)(13) has collected data over a number of years in which he attempts to prove that ascorbic acid is produced in the animal body. Cows fed on diets low in Vitamin C had as high a Vitamin C content of blood plasma, as cows fed on good alfalfa hay, corn silage, and grain mixture, which were high in Vitamin C. Likewise the Vitamin C content of milk, urine and feces was normal. Trials over three or four years showed that the outgo exceeded the intake. So the Vitamin must have been formed in the body of the dairy cow - according to Wallis.
Sutton (10) observed that bulls which had low blood ascorbic acid values and a poor breeding history were usually fed poor quality roughage, and that cows which were fed excellent roughage, had a higher average level of ascorbic acid in their plasma than cows receiving poor quality roughage. Observations made by Phillips (6)(7)(8) tend to favour the latter theory. Any conclusion, drawn from the work of these men is rather confusing, however Vitamin C is likely synthesized some place in the body, but, either the amounts synthesized vary, or the control of the amounts released in the blood plasma is not functioning properly.

The problem of determining the site of the synthesis of ascorbic acid in the body of dairy cattle has baffled all workers. Thurston (11) said that Vitamin C is synthesized within the body of the bovine, and that evidence indicates the digestive tract is not concerned in this synthesis. All the workers agree that Vitamin C is completely oxidized in the rumen but some believe that it could be reformed into the Vitamin farther back in the small intestines. Further work is necessary before the definite seat of formation of ascorbic acid in the body of the bovine can be found.

The role played by ascorbic acid in the physiology of reproduction is not understood. Phillips (6) states that from data now available it appears that ascorbic acid acts in some way to stimulate or 'pep up' the activity of the glands involved in reproduction. Dukes (2) says that ascorbic acid has been obtained in large amounts from adrenal cortex. The prevailing belief is that the cortex stores rather than forms Vitamin C. He also states that there appears to be an important endocrin interrelation of the adrenal cortex and the sex organs. Further, that the adrenal cortex comes under the influence of a factor produced by the anterior pituitary. This gland also has hormonal influence over the gonads. Therefore it seems reasonable that there is an interrelation between the storage of Vitamin C in the adrenal cortex and the control of the anterior pituitary over the adrenal cortex and the gonads.

Very recent literature brings out the theory that there is a close relationship of Vitamin C with Vitamin A, which is in turn as closely related to Vitamin E. This fact helps to explain the administration of both Vitamin C and Vitamin A in the cases of papillary edema mentioned formerly.
During the past fifteen years, considerable literature has appeared, relating sterility and Vitamin C deficiency to dysfunction of the anterior pituitary. Some authors ascribe to the concentrates of Vitamin E, physiological properties resembling those of gonadotropic and esterogenic hormones. Similarly Rosenberg (9) states that ascorbic acid is a hormone for all animals, other than primates and guinea pigs, as they are able to synthesize their own Vitamin C. Further, Rosenberg states that, in the guinea pig, typical changes of the female sex organs occur if a lack of Vitamin C is present. The development of the follicles becomes greatly retarded and no corpora lutea develop at all.

The exact physiological reason for the lack of the ascorbic acid in the dairy animal and the resulting failure to reproduce, has not been determined. Nevertheless it is reasonable to correlate the lack of ascorbic acid in the blood plasma with the hormonal control of the anterior pituitary on the gonads of the dairy animal—especially the corpus luteum of the cow.

One thing is certain—it should be classed as a nutritional disease. Until the tissue or organs responsible for ascorbic acid synthesis are more clearly defined, the cause of impotency due to ascorbic acid deficiency will of necessity remain obscure.

Experimental Data

Injection of the Dairy Bull

Phillips et al. (6)(7) first reported evidence that the blood plasma of cattle fed on restricted rations was low in its ascorbic acid value. The average normal value of the blood plasma ascorbic acid for twenty-two potent bulls with good breeding history, was found to be 0.27 mgs. per cent. Normal range is usually 0.2 to 0.4 mgs. per cent. Following this Lardy and Phillips (7) found that bulls with low fertility show less than 2 mgs. of ascorbic acid for 100 cc. of fresh semen, and in some cases only a trace. Good breeding bulls on the other hand produced semen containing from 3.0 to 8.0 mgs. of ascorbic acid per 100 cc. fresh semen. The method used in calculating the ascorbic acid values in blood plasma and semen is the one used by Mindlin and Butler (4).

A third test is the longevity storage test. Poor breeding or impotent bulls produce semen that will not store in egg-yolk buffer for more than 48-72 hours. Potent bulls
produce semen that will store for periods in the neighborhood of 170 - 200 hours. The storage test is made in fresh hen egg-yolk and buffer, equal parts; and this mixture is diluted with semen in the ratio of one part of semen to three parts of yolk-buffer.

Daily checks on the motility of the semen are made with a microscope (440x). These three tests taken together provide a very accurate estimation as to the potency of a bull.

There is no set therapeutic dosage of ascorbic acid for the dairy animal. This is due to the fact that ascorbic acid cannot be given in large enough quantities to show toxic effects. However, care must be taken in the storage of Vitamin C and in the method of injection. Ascorbic acid is very easily destroyed. In its crystalline form it is fairly stable, so the most practical and most economical way to purchase it is in the crystalline form from chemical supply houses in 1 (28Gms.) or 5 oz. quantities. Once in solution the Vitamin should be used as soon as possible as it oxidizes very quickly when exposed to light and oxygen. The crystalline form should be stored, sealed tightly, in dark colored bottles.

Ascorbic acid can be given per orum to the single stomached animals. In ruminants the rumen contains bacteria that destroy the Vitamin in a very short time. Phillips et al have proven this by feeding the Vitamin in crystalline form and later injecting the Vitamin under the skin. No improvement results while being fed, compared to complete recovery of the bull following institution of subcutaneous injections.

There are three possible solvents into which the ascorbic acid may be incorporated, when ready for injection: (a) a sterilized solution of the buffer used in the longevity storage test of semen can be prepared. It is made up by dissolving 0.1 Gms. (1.5 grs.) of sodium phosphate (U.S.P. dried) in 50 cc. (1.6 oz.) of distilled water. (b) Physiological saline solution. (c) Distilled water.

All three solutions are of equal value but we have found that the most practical method for the veterinarian is to draw up 30 cc. of distilled water in a clean syringe, and inject it into a rubber stoppered brown glass bottle, containing 3 Gms. (46.2 grs.) of ascorbic acid previously weighed out, and placed in said bottles. After shaking until all the ascorbic acid is in solution, the mixture is drawn up in the syringes and administered.

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* "we" refers to my preceptor Dr. H. M. LeGard, “Eston, Ont, and the writer
The 3 gm. dosage is approximately the same as the dosage recommended by Phillips (5)(6). He states that the minimal dose for satisfactory results is 1 Gm. (15.4 grs.) of ascorbic acid per 454 kg. (1000 lbs.) of body weight. Roughly it should be given at the rate of 2 - 4 Gms. per animal, twice per week. Larger doses seem to be unnecessary. The treatment can be continued for five or six weeks, or until the bull shows improvement.

Phillips (7) tells of one bull whose semen contained no ascorbic acid prior to treatment. After 2 to 3 weeks the ascorbic acid content of the semen reached the normal level of approximately 6.19 mgs. per 100 cc. fresh semen on the average. The appearance of ascorbic acid in the semen was accompanied by distinct changes in the character of the semen sample. Formerly the semen was thin and watery and totally devoid of life. After treatment it was normal in appearance, thick and creamy with every evidence of life and vitality. Heifers conceived after being bred with the semen stored for 96 hours.

Some bulls showed a lack of sex interest and a few would not breed at all. Following vitamin C therapy there was a distinct difference in sex interest and they returned to service.

On the average, following the inauguration of the therapy, there is reported (7) a 95 per cent rise in the ascorbic acid content of blood plasma. The ascorbic acid content of the semen rose from less than 2.0 mgs. to between 3.0 to 8.0 mgs. for 100 cc. fresh semen. Coupled with that, the longevity storage test of the semen showed much higher records.

It is interesting to note that one Guernsey bull which failed to respond to ascorbic acid treatment, was given large quantities of Vitamin A. The ascorbic therapy was continued and the bull soon returned to the production of high grade potent semen.

Some bulls show improvement after a few weeks of treatment and then have a relapse when returned to heavy service. A second treatment was of benefit in these cases.

Although we had the opportunity to treat only two bulls, in both cases the treatment was successful. In one case the bull was a young mature animal which had been overworked. The ascorbic acid content of the semen was not tested either before or after treatment but both the character of the semen and the longevity storage test showed a marked improvement.
following treatment. Besides he began to settle the cows bred to him both naturally and artificially. It is well to remember that not all impotent bulls are successfully treated. Phillips (6)(7) however reports 60 to 75 per cent of the cases respond to the Vitamin C therapy. Research indicates that two kinds of sterility in bulls can be successfully treated with ascorbic acid. The most favourable results are obtained when bulls are in a growing and developmental process, and in other cases where frequently used and potent bulls have begun to decline in their ability to serve and settle a cow.

Injection of the Dairy Cow

Following the treatment of sterile bulls Phillips and his associates turned to the 'hard to settle' cows. Analysis for blood plasma ascorbic acid were made in both the normal cow and the cow with a poor breeding history. He found an average value for ascorbic acid content of cow blood was approximately 0.39 mgs. per cent with the range of values lying between 0.19 and 0.65 mgs. per cent. These results compare favourably with the results of Bortree and associates: (1), where 356 samples were taken of 24 normal cows showing a mean of 0.44 mgs. per cent of plasma (range 0.11 to 0.80 mgs. per cent). However Knight and co-workers (3) reported higher values, while Wallis (12) reported lower values. Considering the results of the reports there is quite a variation in the ascorbic acid content of bovine plasma from day to day and even from hour to hour.

Beside the variation of individual cows there is also a variation among breeds. In general the Guernsey breed requires higher concentrations of ascorbic acid in the blood than the Holstein. Comparing the average values of the cow and the bull in the same breed the cow requires approximately 0.1 mg. per cent more ascorbic acid in the blood plasma for proper reproduction.

Perhaps the most significant piece of evidence found was the difference of the ascorbic acid content of blood plasma at diestrum and at estrum. In most cases cows with a regular and consistent breeding history showed a rise of as much as 95 per cent of ascorbic acid in blood plasma. The peak was reached in mid to late estrum. In cows with a poor breeding history there was corresponding rise during estrum. Phillips (8) cites a case which brings this out very plainly. This cow calved normally and 9 weeks later began to have normal heat...
periods at 21 day intervals. Ten such periods followed at which she was bred 8 times. When her blood was analyzed the values were 0.54 mgs. per cent at diestrum and 0.51 mgs. per cent ascorbic acid at estrum. At her 11th estral period ascorbic acid was injected and she conceived to artificial insemination the same day.

Cows which skip one or two estral periods, and come back in heat on the 42nd. or 63rd. day, are good subjects for ascorbic acid therapy. These cows have been slaughtered at the 24th. day and it was found that a degenerating or cystic corpus luteum has been the cause. Since these cases respond to the treatment as well as the cows with the regular heat periods it is reasonable to surmise that the corpus luteum may be at fault.

For a number of years a valuable Guernsey herd had been troubled with sterility problems. Manual manipulations had been used, with varied success. These were usually a small percentage of high producing cows which showed no abnormalities in their reproductive tract, but just would not settle. The cows were given a rest on pasture, and then pasture breeding was attempted. Artificial insemination was also tried but with little success. In August 1944, 17 cows that showed a poor breeding history, and with no noticeable anatomical abnormality throughout their reproductive tracts, were singled out for ascorbic acid treatment.

For economic reasons the injections were limited to one injection on the day the cow was in estrum. 3 grams (60,000 I.U.) of ascorbic acid were mixed in 30 cc. of distilled water and injected after midestrum and an hour before submitting the cow to service. Instead of administering the 30 cc. subcutaneously, 10 cc were injected into the jugular vein, and the remaining 20 cc. were given subcutaneously. It was felt that the ascorbic acid concentration in the blood would be at a peak when it was necessary for the stimulation of the corpus luteum (Phillips (5) advocates the administration of 2 Gms. intravenously and 2 Gms. subcutaneously at estrum followed by subcutaneous injections twice a week for three weeks).

Discussion

From the observations made from the data it seems that ascorbic acid therapy can be carried on successfully in the field. It is true the cows were treated under ideal conditions,
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Note - Type of Case -

A - Regular Estral Period and Failed to Settle

B - Skips 1 or 2 Periods After Service.

C - No Tone in Uterus.
and there was good cooperation between herdsman and veterinarian. The cattle had just completed a summer rest, which fact alone might well have changed the picture regardless of the vitamin therapy. However, it must be noted that a potent bull was running with the cows for three months before the cows were treated and bred.

Under field conditions it was impossible to use a check test; neither did we try animals that had cystic ovaries or any other anatomical abnormality. The cows treated have not calved as yet but they have all missed three or more estral periods. The cows that required more than one treatment might have settled after the first breeding if subcutaneous injections had been continued twice a week for several weeks.

The treatment was adopted with the idea of raising the concentration of ascorbic in the bloodstream, as in cows with normal breeding histories. This we thought would give the corpus luteum the required ascorbic acid it needed in its development following ovulation.

Of particular interest was cow No. 1043. She had been bred 13 times prior to treatment. After one injection followed by service she conceived. Naturally it could be suggested that she would have conceived without treatment. However it should not be overlooked that 9 out of 17 treated cows conceived after the first treatment.

Phillips (5) (8), claims that cows should be treated a number of times following the initial dose. This may be of advantage but is not practical in the field. Comparing the results in the field and those derived experimentally, there is only approximately 5 per cent difference in the average of responses attained.

Anatomical abnormalities have never been corrected by ascorbic acid therapy. The cases which do seem amenable to this type of therapy apparently fall into the following classes:
(a) Cows with regular heat periods, and whose reproductive tract upon examination appears normal.
(b) Cows that apparently settle on service and skip the next heat period or two before coming into heat again. Because of the high concentration of ascorbic acid in the corpus luteum and its functions in pregnancy, it is thought that these cases are involved in an
REFERENCES


