EFFECT OF THE ORAL ADMINISTRATION OF CHLOROBUTANOL ON REPRODUCTION OF THE MALE AND FEMALE BOVINE

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INTRODUCTION

The problem of reproductive difficulties with both the male and the female bovine has long been one of concern to many dairymen. With the advent of the proved sire and the artificial insemination programs, the average age of sires used in dairy cattle breeding is constantly increasing. In one dairy herd improvement association in Michigan in 1943, the author found that twenty-five per cent of the sires became impotent. In some cases it was due to the sire losing his libido, in other cases it was due to the low motility and fertility of the sperm.

All the problems of sterility are not confined to the males. In 1940 Baltzer (13) reported that over a five year period in Michigan seven per cent of the cows on test in dairy herd improvement association work were removed due to sterility.

The use of ascorbic acid by subcutaneous injection as a cure for bovine sterility was reported by Phillips and co-workers (5), (14), (24), (26). Additional evidence that ascorbic acid aided breeding performance in livestock was reported by Davis and Cole (3) in their work with horses. The work of Bortree, Huffman and Duncan in 1941 (15), (16) showed that the synthesis of vitamin C in dairy cows and bulls could be stimulated by orally administering chlorobutonal. Since that time Lundquist and Phillips (31) have also reported the positive effect of increasing blood plasma ascorbic acid values by feeding chlorobutonal. In view of the relationship of ascorbic acid metabolism to reproductive efficiency, it seemed advisable to study the effect of chlorobutonal administration to sterile cows and bulls.

REVIEW OF LITERATURE

The problem of reproduction in dairy cattle divides itself naturally into two fields of study, namely: (a) that of the males and, (b) that of the females. Jones and co-workers (48) enumerate the reasons for poor reproduction in males and females, namely, inadequate nutrition; poor management practices; disease conditions; inheritance of low fertility factors; and disturbances of the endocrine gland system which in turn is affected by nutrition, heredity and disease.

The literature pertaining to libido and semen quality in the male and failure to conceive in the female will be reviewed.

Reproduction in Males

Reproductive failure in the male is due either to a lack of libido or infertility of the sperm.

Libido

The problem of improving the libido of sires is rarely mentioned in the studies of sterility in the male bovine.

Nutrition factors

Phillips (5) in his studies with sterile bulls observed that their sex drive improved with an increase in the blood plasma ascorbic acid values.

Loptev (83) reported that green feeds are valuable in stimulating sex drive. The feeding of green grass, or grass silage, or 4.7 pounds of beet fodder per 100 pounds of body weight stimulated the sex drive within about 15 days.

More recently Albrecht (73) reported a marked difference in the libido of rabbits fed two kinds of lespedeza. Male rabbits fed for three weeks on lespedeza hay grown on soil fertilized with phosphorus refused to mate with does in estrum. Those fed lespedeza hay grown on soil fertilized with both lime and phosphorus continued to be sexually excited by does in estrum. Males on the hay from phosphorus treated soils returned to normalcy when fed hay grown on soil treated with both calcium and lime.

Vitamin E did not play a role in improving the libido of twenty sires fed wheat germ oil as reported by Salisbury (88). He stated that the service time was the same as the control group which was 104 seconds per ejaculate.

Timin and Perelurian (85) reported improvement in the libido of bulls fed 800 to 1000 grams of wheat germ per bull per day.

Boyer and co-workers (51) reported that rats on a manganese deficient diet lacked sex interest. Roberts and Dawson (49) found that the feeding of arsenic solution to rabbits decreased libido considerably. Only 4 per cent lacked libido in the control group, while 39 per cent in the experimental group lacked sex desire.

Hormones

Moore (27) stated that one important function of the hormones secreted by the testis is that of regulating the behavioristic reaction of the male as evidenced by sex drive. He also mentioned that the pituitary gland is the master regulatory organ. Turner (46) stated "that low thyroid activity could cause bulls to become sluggish in mating." He reported a marked improvement in libido of bulls fed thyro-protein (thyroid active casein) for a few days. This worker also pointed out that the effect of the thyroid feeding may be indirect since the thyroid gland influences the pituitary gland.

The importance of the thyroid in maintaining the libido of sires has also been reported by Peterson and associates (43) and Spielman and co-workers (94). There was complete inhibition of libido in thyroidectomized males. These investigations and that of Turner definitely indicate that when the thyroid of the male has become temporarily or permanently non-functional, one can expect the absence of libido. Turner stated, "that this is due to a lowered metabolic rate."

Stone reported (18) that castrated rats which had lost all power of libido were rejuvinated by subcutaneous injections of the male sex hormone in the form of testosterone propionate.

Libido has no relationship to conception rates according to Donham and co-workers (28) and (89). In 105 matings using bulls 75 were recorded as prompt. These resulted in 40 per cent pregnancies. The thirty services that were reported slow resulted in 50 per cent pregnancies.

<u>Exercise</u>

Kelley (80) found that with slow bulls exercise greatly diminished the time of service.

It is evident from this review of literature that there is a need for more information concerning the factors affecting libido in the male.

Factors Affecting Quality of Semen

The quality of semen produced will determine to a large extent the breeding efficiency of the sire. Many factors play an important role in affecting the quantity and quality of the semen produced.

Age of sires

Whether the age of the sire has any bearing on the quality of the semen and the rapidity of service, has received some study during recent years. Evidence that older bulls can stand heavier service was reported by Phillips (5) in which he concluded that junior yearling bulls can easily be over-used causing them to produce low quality semen. He recommended one service every seven to ten days for a bull of this age. With older sires Phillips suggested one service every fourth day in order to maintain a high quality of sample. Heider and co-workers (4) using data from 8,919 services report that the service per conception for bulls of all ages was 2.67; bulls 7 to 10 years of age required 3.14 services per conception, while bulls under 5 years of age 1.52 services per conception. Bowling and associates (1) in studying breeding records over a period of 18 years found that bulls thirteen years of age or over were significantly less sure breeders than bulls six years of age. Their tabulated data are as follows:

Age of Sire	Services Per Conception
1 - 2 years	1.50
4 - 5 years	1.56
7 - 8 years	2.05
10 - 11 years	2.10
14 - 15 years	2.78

On the other hand Baker and associates (2) from studies with range beef bulls reported that the age of bulls did not have a significant effect on the calf crop. No report was made on the number of services per conception. It is possible that the results might have paralleled those for dairy cattle.

Lethal factors

It is apparent that lethal factors can reflect themselves in the breeding of females according to work done with cattle by Mead et al. (6) who reported that two forms of sterility are sex-limited.

Season and climate

During the past four years several rather comprehensive reports have been made on the effect of season and climate on the semen of sires. Two of the reports that support the theory that the best semen is produced in the spring and the poorest in the late summer come from states in the same latitude. Phillips et al. (82) reported that total sperm produced was highest in spring and lowest in the fall. In the examination of the results of 1,135 matings they found that the highest percentage of fertile matings occurred in April with 59.6 per cent and the lowest was in August with 40.8 per cent. Storage life of the semen in the summer time was 102.7 hours, that in the winter time 126.7 hours. Other months showed little seasonal effect on the survival in storage. Erb and co-workers (11) as a result of a study of 879 ejaculates produced by nine bulls over a period of one year expressed the belief that temprature changes are at least partially responsible for the differences in the semen quality. Their studies confirmed those of Phillips et al. (82) who found that semen of consistently inferior quality was produced during July, August and September, while semen of superior quality was produced in April, May and June. Season of the year also appeared to influence the percentage of abnormal spermatozoa. The number of abnormal spermatozoa was 25 per cent greater during July, August and September than for the next highest month of the year. Similar results were reported by Swanson and Herman (91). They concurred with previous workers in that the highest number of abnormal sperm was in summer. Their work is of interest in that young bulls were not affected as much by season as were older bulls. Swanson and Herman also found that the pH of semen was significantly lower in the summer than in the fall. These workers concluded that spermatogensis in dairy bulls is not greatly affected by season.

Salisbury (10), and Weatherby and associates (58) did not find a great variation in semen quality because of season of year. Anderson (75) reporting on semen studies in England found that poorest semen production was in May, June and July. This is exactly contradictory to the findings on this continent.

Frequency of use

The quality of the semen of many sires has probably been lowered considerably by too frequent service. Weatherby and associates (58) found the collection of ejaculates once a day for 57 days from a two year old Guernsey bull, that the concentration of the sperm changed considerably and that the average longevity was only four days. When changed to one ejaculate once a week the volume increased from 2.6 to 4.1 cc and the longevity to 12 days.

Exercise

Bartlett and Perry (72) reported that bulls exercised for one hour at two and one-fourth miles per hour resulted in increasing the volume of semen by 51 per cent. Kelley (80) found that exercising bulls at a speed of two and one-half miles per hour improved motility from a range of 5 to 45 per cent to 60 to 100 per cent. Life of the sperm was 3 to 24 hours for bulls with no exercise compared to 22 to 39 hours for those with exercise.

Ration and Semen Quality

Vitamin C

Phillips and associates (14) reported that bulls having a low ascorbic acid value for semen (two mg. per cent or less) were brought back to normalcy in a large majority of cases in five weeks' time by the subcutaneous injection of vitamin C. Phillips also pointed out that semen ascorbic acid values above eight mg. per cent were toxic and the quality of the semen may be as poor as when ascorbic acid values are low.

Just how vitamin C improves the quality of semen is not definitely known. Several theories have been advanced. Berg (32) and co-workers found that the ascorbic acid is concentrated in the seminal vesicle. Thus one might assume that the spermatozoa are kept viable after spermatogenesis is completed by the ascorbic acid in the seminal vesicles until they are ejaculated.

It is generally conceded that young sires have a higher quality of semen than older bulls. This phase of the problem was reviewed earlier by Heider (4) and Bowling (1). A possible logical explanation of the higher quality of the semen of young bulls is given in the work of Biskind and Glick (34) who found that in bovine testicles the vitamin C reaches a maximum concentration soon after birth.

Di Cio and Schteingert (45) using two to four months old white rats found a favorable effect of vitamin C on the development and function of the gonads. The injection of 50 mg. of ascorbic acid and 25 rat units of gonadatropic hormones per day for a period of twenty to thirty days resulted in twice the average penis weight than when the hormones were used alone. The testis increased in size at the same rate as the penis. The weights of the testis for the four different groups were as follows:

Group	Wt. in gms. of testis
1. Normal	0.361
2. Ascorbic acid alone	0.377
3. Gonadatropic alone	0.672
4. Ascorbic acid - gonadatropic	1.150

Simultaneously with the report of Di Cio and co-workers, Erb and Andrews (44) reported the effect of injecting 2250 rat units of gonadatropic hormone into a bull. There was a decrease of 42 to 67 per cent in the venous blood plasma ascorbic acid values during the first twenty-four hour period. About two weeks were required for the plasma ascorbic acid values to return to normal following the injection. Almquist and Andrews (42), however, in experiments where the simultaneously injected 50 mg. of ascorbic acid and 25 rat units of the gonadatropic hormone in 24 day old rats for four days and in 70 day old rats for 20 days produced no greater testis weight than 25 units of the hormone alone. They concluded that there would be no effect on rats over 50 days of age since they produce complete spermatozoa and are sexually mature at that age.

Reid and Sykes (54) working with guinea pigs obtained the same results with the males, as Almquist and Andrews did with male rats. They reported no augmentation in testis weight when 50 mg. of ascorbic acid was added to the basal diet of rats that had received injections of the gonadatropic hormone at the dosage of either 50 or 100 rat units.

Vitamin C may indirectly affect semen production by affecting the plane of nutrition. McHenry (99) and co-workers working with guineapigs discovered that on vitamin C deficient diets, appetite disappeared and eventually death occurred. They found that appetite could be maintained with as little as one-tenth of a milligram of ascorbic acid per guinea pig.

Some factors influencing vitamin C synthesis

The value of sunlight and vitamin C synthesis was noted by Umsva (37). He believed that solar radiation stimulated the organic synthesis of ascorbic acid in man and animals. The work of King and associates (100), (101), (102) with rats showed that the feeding of several compounds stimulated the synthesis of ascorbic acid. The feeding of carvone, chlorobutanol a lipid fraction from alfalfa, and many other compounds with a low ascorbic acid diet increased urinary excretion of this vitamin. Bortree et al. (15) showed that the feeding of chlorobutanol to cattle resulted in a marked increase in plasma ascorbic acid. Recently Linquist and Phillips (31) have shown that succinylsulfathiazole fed at the rate of 2.0 gm. per day to new born calves raised the blood plasma ascorbic acid values considerably during the first week. Succinylsulfathiazole when fed at the rate of 15 gms. daily to cattle increased blood plasma ascorbic acid values similar to chlorobutanol.

Chlorobutanol has a phenol co-efficient and could retard the development of bacteria in the rumen. Linquist and Phillips (96), however, showed that it had no injurious effect on the synthesis of thiamine, riboflavin, niacin and pantothenic acid when fed at a level of 5 gm. per thousand pounds of liveweight for 160 days.

Inter-relationship of vitamin C and other vitamins

It has long been known that there is definite vitamin inter-relationship in various animals. Sure and co-workers (39) using rats concluded that one avitaminosis produced on a diet satisfactory in every respect leads to the exhaustion of reserves of other vitamins. Their report on individual and multiple vitamin relationships to ascorbic acid was as follows:

1. Vitamin B deficiency caused a decrease of vitamin C in various tissues as heart - 35 per cent; kidney - 23 per cent; liver - 19 per cent and thymus - 27.8 per cent.
2. Vitamin A deficiency decreased vitamin C content of heart - 20 per cent; kidney - 27.3 per cent and thymus - 24.1 per cent.
3. Vitamin B_6 deficiency - no noteworthy change in vitamin C in tissues or endocrines.
4. Multiple depletions results in a vitamin C loss of -

   26.6 per cent in the kidneys;
   45.0 per cent in the liver;
   19.2 per cent in the thymus;
   12.4 per cent in the adrenals;
   25.8 per cent in the pituitaries;
   15.3 per cent in the thyroids.

Kimble and Gordon (98) reported that the administration of riboflavin and vitamin A failed in some cases to improve the plasma vitamin values and the biophotometer performance. While these values were improved by the administration of ascorbic acid, according to Belasco and Murlin (33), the use of either vitamin A or C as a supplement reduced the hypermetabolism associated with hyperthyroidism. They stated that the reduced hypermetabolism was associated with an increased respiratory rate of the liver and the kidney cortical tissue.

Phillips and co-workers (38) (5) (14) showed that blood plasma ascorbic acid declines with the development of vitamin A deficiency. Twenty cubic centimeters of cod liver oil per day was required to maintain normal ascorbic values in Guernsey bulls.

Sutton et al. (35) fed young bull calves on a low vitamin A ration to one year of age. The postmortem findings were as follows: degeneration of the germinal epithelium of the testis, absence of spermatozoa in the epididymis, and an accumulation of fluid in the cleft between the anterior and posterior lobe of the pituitary gland. Wolback and Bessey (20) were of the opinion that the atrophy of the seminiferous tubules in vitamin A deficiency as in other epthelial organs spares the undifferentiated cells and hence recovery is possible with replacement therapy. Sutton (36) working with white rats found that a restriction of vitamin A caused a marked decrease in the blood vitamin C. Erb and associates (92) reported that a bull fed a low vitamin A ration for four months went blind, had staggers, and gastro-intestinal disturbances. By using testicular biopsy they found severe degeneration of the seminiferous tubules and nearly complete disappearance of sperm from the lumena of the tubules. Feeding of 60,000 to 150,000 international units of vitamin A per day resulted in recovery within two to five months. Just before semen production ended, the number of abnormal sperm was four times that of a healthy bull.

Guilbert (95) in his review of studies of nutritional reproductive failures also mentioned testicular degeneration due to vitamin A deficiency. Phillips (68) reported that cellular sloughing is noted in vitamin A deficiency but spermatogenesis continues. The condition could also be repaired after 60 to 90 days of vitamin A administation.

More recently Moore and Cotter (97) in a well controlled experiment with young bulls seven to fourteen months of age and with older sires studied the effect of vitamin A intake on plasma ascorbic acid values. They concluded that only in extreme vitamin A deficiencies would there be a decrease in the amount of vitamin C excreted per day or a depression in the blood plasma values. They predicted that extremely high levels of vitamin A intake might stimulate vitamin C synthesis. They further stated that it remains to be shown that low carotene intakes of animals fed normal winter farm rations affects vitamin C synthesis or results in breeding deficiencies of sires.

Vitamin E

That vitamin E has no value in improving semen quality has been reported by Gullickson and co-workers (87). They fed seven males on a ration devoid of vitamin E and found that growth, physical and sexual development were normal in every respect. In the work of Salisbury (88) sires fed vitamin E in addition to the normal ration failed to show an increase in the volume of semen, sperm concentration or motility of the sperm.

Timin and Perelurian (85) on the contrary reported decidedly favorable results from the feeding of vitamin E, in the form of wheat germ concentrate. These workers in comparing five bulls fed the concentrate to five bulls fed the basal ration reported a 31.5 per cent increase in spermatozoa volume, 63.0 per cent increase in sperm viability and a 32 per cent increase in sperm concentration in favor of the wheat germ. The beneficial effects were noted 25 to 30 days after feeding started. They recommend 800 to 1000 gms. per bull per day.

Amino acids

Whether dietary protein has been a factor in producing high quality semen has been under investigation for some time. In studying sterility problems Cunningham and Hopkirk (22) found that feeds which were too high in protein content brought on sterility. Using rats, the feeding of protein diets of 65 to 82 per cent and those containing 15 to 18 per cent protein from maize and gelatin caused sterility, which was associated with a degeneration of the testis. These results were believed to be due to an amino acid deficiency. Webster (23) reported a high evidence of sterility and abnormal spermatozoa when the crude protein of the grass was as high as 35 per cent on a dry matter basis.

Holt and co-workers (50) working with humans found that a diet deficient in arginine over a ten day period caused a reduction in the number of spermatozoa to one-tenth of the normal values. Apparently atrophy of the spermatogenic tissue took place. A definite standard for a ration for breeding bulls has been worked out by Pankevic (74). He stated that a sire should get 411 gms. of digestible protein for each 500 kgs. of body weight; furthermore the feeding of the animal at the rate of 162 gms. of protein per mating should keep the bull in good breeding condition. The use of proteins of animal origin in the ration of sires was made by Laptev (83) and Smernov-Ugrjunov and Laptev (21). The use of blood meal and skimmed milk caused a makred improvement in the sperm concentration. These feeds apparently influenced spermatogenesis since a noticeable effect took place in 17 to 37 days. Concentrates of plant origin rated next to the two proteins of animal origin. They mentioned that green grass alone caused a decrease in the amount of the ejaculate.

Dairymen in many regions have turned to alfalfa as the sole source of hay. This legume is low in sulphur containing amino acids. Jones and associates (48) using two groups of bull calves starting at 5 to 7 months of age and continuing to 32 to 41 months of age compared a ration of alfalfa hay, salt and disodium phosphate, to one of alfalfa hay, salt, disodium phosphate and a grain mixture of skimmilk powder and oat groats. In comparing the initial motility of the semen of the two groups it was found that the alfalfa hay group had only 52 per cent of the semen score excellent, compared to 72 per cent for the supplemented hay group. Both groups of bulls, however, were fertile. The bulls fed hay alone required only 1.27 services per conception compared to 1.54 for bulls of the supplemented group. The Jersey bulls produced 3.75 cc of semen per ejaculate compared to 6.39 for the Holstein bulls.

Guilbert (95) stated that when feed restriction takes place which retards growth in the young or weight loss in adults, testicular atrophy may occur in the male.

Minerals

Lardy and associates (52) fed a group of bull calves to 18 months of age on a grain ration containing only 28 parts per million of manganese. The semen produced was not as high in quality as that of the ration high in this element. They mentioned, however, that most farm rations are higher ih manganese than the one they fed. Johnson (47) has shown that with pigs reproduction was not successful with one-half part per million of manganese. Supplementing the ration to bring the manganese content to six parts per million brought back normal reproduction.

Boyer and associates (51) in investigating the effect of manganese deficient diets in the rat concluded that it did not reult in a lowered ascorbic acid content of the various tissues. However, the rats on the deficient diets showed complete lack of spermatids and spermatozoa. The nuclear division was decreased or absent resulting in a decrease in the size of the individual tubules. The lumen of the tubules was filled with degenerating cells instead of spermatozoa. These workers also noted that ascorbic acid feeding had no growth stimulating powers when added to low manganese diets.

That iron is present in spermatozoa was reported by Zittle and Zitin (29). They reported that the non-hemin iron of bull spermatozoa was estimated at sixty per cent of the total iron.

In an extensive study using three thousand rabbits Roberts and Dawson (49) found that arsenic fed in the form of Fowlers solution had injurious effects on the reproductive system. The treated males produced less semen and had fewer sperm; however, there was no difference in the motility of the sperm. There were also fewer young in the litters sired by the treated males.

The sulphur content of sperm is quite high and varies from 1.5 to 1.8 per cent according to Zittle and O'dell (77).

Hormones and semen quality

Many investigators have mentioned the fact that the pituitary gland plays an important role in reproduction. Phillips (14) stated that this gland plays a role in normal reproduction in the bovine. Walton and co-workers (25) and Phillips (68) found that spermatogenesis dependent upon normal functioning of the anterior pituitary. In fact the removal of the gland caused spermatogenesis to cease.

That the thyroid has no effect on the quality of the sperm has been effectively demonstrated by Peterson and others (43) and Spielman and associates (94). In both reports it was stated that normal reproduction took place from the semen of thyroidectomized males at four months of age. The gonads developed normally and there was no deleterious effect on the volume of semen, longevity and fertilizing ability of the sperms. It was also found that the blood plasma ascorbic acid values were normal.

Drill (64) found that in hyperthyroidism there is an increased requirement for vitamin C.

Characteristics and preservation of spermatozoa

Morphology

The relation of the morphology of the sperm to fertility has been studied by Swanson and Herman (12). They found that coiled tails and pyriform heads did not reduce fertility up to 30 per cent of the total volume of sperms. Salisbury and associates (60) (81) showed that the methods of cooling influences the morphology of the sperm. These workers found that in order to avoid injuring the vitality of the sperm that it was necessary to cool semen by reducing the temperature at a rate of 5° C. every 10 minutes to a storage temperature of 5° C.

They also report that the rate of warming to bring back motility should be rapid. In fact, thirty degrees per minute was not harmful. Furthermore, Salisbury and Mercier (90) found that to determine accurately the number of morphologically abnormal sperm that only 100 needed to be counted on a slide.

Ely and associates (70) reported that a large number of abnormal sperms in semen is an indication of low quality as judged by oxygen consumption. Beck et al. (65) devised a method to quickly evaluate semen low in oxygen consumption.

Studies by Dougherty and Ewalt (78) found that there was very little correlation between the abnormal forms and the fertilizing ability of bulls. They found that crooked tails were the principle type of abnormality. Lasley and Bogart (71) in making a careful study of the location of the bending of the tail found the bending was at the position of the protoplasmic droplet on the posterior region of the midpiece.

Air and sunlight

Both MacLeod (79) and Letard (67) pointed to the fact that semen cannot be exposed to air and sunlight very long without killing the sperm. Accame and associates (9) working with rams in high altitudes concluded that the altitude does not affect the spermatozoa but probably has some detrimental effect on the reproductive system.

pH and sperm concentration of semen

Salisbury (59) commented on the fact that the hydrogen ion concentrate is of some value for estimating the quality of semen. He stated that the pH of the semen ranges from 6.0 to 7.2 with a mean of 6.5. Davis and Williams (56) reported pH values for ejaculates taken in succession. The first, second and third ejaculates had pH values of 6.85, 7.23, and 7.21, respectively.

Salisbury (59) stated that the number of sperm usually varies from 300,000 to 1,700,000 per cubic m.m. and that good quality semen should have 600,000 or more sperms per cubic m.m. Davis and Williams (56) reported concentrations of 826,000 per cubic m.m. for the first ejaculate, 635,000 for the second and 337,000 for the third. Salisbury and Davis and Williams used the hemo-cytometer for sperm counts. Schaffner and Andrews (62) developed a method of counting sperm using hematocrit tubes since in using the hemo-cytometer one out of nine samples varied in count as much as twenty per cent. They obtained more uniform results with their method and gave an average count of bovine semen as 950,000 sperms per cubic mm.

Storage of semen

Semen can be stored either with diluter or undiluted. Underbjerg and Davis (57) found that the motility of diluted and undiluted semen was maintained for 200 hours. Furthermore they reported that the egg yolk salts of diluters had little effect on the fecundity of stored samples.

Most investigators are in agreement on the temperature to use for storing semen. Davis and co-workers (66) found that 35° F. gave a better storage record than temperatures of 40° or above. Salisbury (59) found in his work that the best storage temperature was 5° C. which is close to the temperature recommended by Davis and his associates.

Standard of semen quality

Several workers have attempted to establish standards to use in estimating the quality of semen which is satisfactory for normal conception values. Swanson and Herman (93) using 23 sires reported that semen to be of good quality should have a progessive motility of 45 per cent or more.

Lasley (69) corrleated the motility and fertility of bull sperm. In samples with less than 40 per cent motility the fertility was 54 per cent compared to 69.4 per cent when the motility was 61 to 70 per cent. In comparing the quality of semen of the first and second ejaculates, Davis and Williams (56) found that the first ejaculate required 1.33 services per conception and the second ejaculate 1.25 services.

Another standard of motility for age can be taken from the work of Davis and co-workers (66). Their results with storage of undiluted semen at 35° were:

Storage Age	No. of Samples	Motility
Fresh	47	81 per cent
24 - 28 hours	12	60 per cent
48 - 52 hours	33	53 per cent
72 - 78 hours	10	47 per cent

Reproduction in Females

Several workers have published data which give the livestock breeder a standard with which to measure reproduction in the female bovine. Heider and co-workers (4) reported that with dairy females of all breeds there was little apparent effect of age upon breeding efficiency up to nine years of age. They also found that breeding cows for a 365 day record instead of a 305 day record did not decrease the breeding efficiency. Using 432 cows in this study, they reported that the cows bred to calve at 15 months interval required 2.54 services per conception compared to 2.69 services per conception for those bred for 12 months calving interval. They also recommended that in commercial herds cows do not warrant more than five services. Comparing heifers bred for the first time and older cows, they found that the heifers required 3.23 services per conception compared to 2.76 for the older cows.

Similar data showing that virgin heifers do not conceive as readily as older cows was reported by Bowling and collaborators (1). They reported that the number of services required for conception were as follows: first 2.79; second 1.86; third 1.79; fourth 1.82; fifth 1.80, and for all services during life time 2.02. They used 706 females in this study. Working with beef cattle Rhoad (8) concluded that there was not much difference in the rate of conception for heifers and cows.

Factors Affecting Conception

Libido and conception

Unless cows have regular estrus periods conception cannot take place. A report by Pinetem (84) stated "that the feeding of ergot of rye had aphrodisiacal powers." By feeding thirty grams to cows and sows after fasting the animals came in heat in a few days. If they were not successful the first time the dose was repeated. Successful results were obtained from sixty to one hundred per cent of the time with cows.

A lack of sex interest was produced in female rats by feeding a ration low in manganese according to Boyer and co-workers (51).

Lethal factors and conception

Mead et al. (6) using six bulls tested for deleterious recessive genes which were associated with female sterility. Two different forms of female sterility that prevented heifers from conceiving or producing offspring were found. All the affected animals in the Jersey herd manifested normal heat periods. The sterility found in the Holstein breed was associated with abnormal estrous cycle, usually complete absence of heat.

Nutrition and Conception

Vitamin C

According to Riddell and Whitnah (40) and Wallis (41) cows synthesize sufficient vitamin C to supply their needs. Phillips and co-workers (24) reported that occasionally cows do not synthesize sufficient quantities of vitamin C and consequently show a reduced conception rate. They found that "hard to settle" cows did not show an elevation of ascorbic acid values during estrum compared to normal cows which showed a 75 per cent increase.

The treatment of sterility by subcutaneous injections of ascorbic acid resulted in conception in 60 per cent of the cows.

That vitamin C is concentrated in the corpus luteum was reported in studies of humans by Ley (30). He treated 10 women who had experienced two or more abortions by the following procedure: early in the subsequent pregnancy each women was given injections of vitamin C until the blood was saturated; then smaller doses were given to the end of the sixth or seventh month of pregnancy. All ten women delivered living children.

Biskind and Glick (55) working with cows were of the opinion that the corpus luteum is physiologically related to the anterior lobe of the hypophysis and that it contains vitamin C in approximately the same concentration. They made determinations of the vitamin C content of the corpus luteum of cows at various stages of the estrus cycle. They found that when the organ was fully developed that there was 1.4 mg. of ascorbic acid per gm. of tissue and following regression it contained only 0.3 mg. per gm. During gestation the vitamin C level remained at 1.5 to 2.2 mg. per gm. of tissue for seven months and decreased to 1.1 gm. in the eighth month. The authors thought that there might be a relationship between vitamin C and progesterone.

Studies have been conducted by Erb and associates (44) on the effect of the gonadatropic hormone on the blood plasma vitamin C of cows. The injection of 2250 rat units of the hormone caused a 20 to 50 per cent decrease in the ascorbic acid values during a 24 hour period.

Almquist and associates (42) working with rats failed to find any effect of ascorbic acid administration on the gonadatropic hormone. There was no increase in the size of the ovaries or the uterus. Sutton and coworkers (17) reported that the estrogenic hormone caused an increase of vitamin C in both the blood and the urine.

Working with female guinea pigs Reid and Sykes (54) were able to potentiate the injections of gonadatropic hormones by adding 50 mg. of ascorbic acid to the basal diet. The weight of the ovary was 1.38 times that obtained when gonadatropic hormone was added alone.

Vitamin A

The effect of the daily intake of vitamin A in relation to conception rates was studied by Kuhlman and Gallup (53). They found that the minimum daily carotene requirement of dairy cows for normal reproduction appeared to be 40 to 45 micrograms per pound of body weight. The conception rates of cows fed different levels of carotene intake were as follows:

Days Fed Before Service	Number of Cases	Carotene Intake	Services Per Conception
90	21	20-39 micrograms	1.99
90	23	40-59 "	1.35
90	15	60-99 "	1.15
90	15	100-353 "	1.21

Moore and Cotter (97) feeding dairy heifers on a carotene free grain mixture, 10 pounds of wheat straw and carotene in alfalfa meal equivalent to 42 micrograms per kilo of body weight, found no particular relationship between the stage of gestation and the excretion of ascorbic acid. Five grams of chlorobutanol fed to two of the heifers resulted in four to eight fold increased ascorbic acid excretion in the urine. The ascorbic acid values of the blood of pregnant heifers fed a low carotene ration did not increase, however, they excreted large quantities of it.

Guilbert (95) stated "that vitamin A deficiency results in death of the fetus or the birth of nonviable young."

Bortree (61) reported that a four year old heifer receiving only 14 micrograms of carotene per pound of body weight for a considerable length of time had vitamin C values within the normal range. He observed that animals on a low vitamin A ration did not have a wide variation in vitamin C values.

Vitamin E

The effect of vitamin E on conception was studied by Henke (86). Thirty-eight cows with irregular breeding behavior were fed sprouted oats. Of these 82 per cent produced calves. Thirty-seven cows with similar irregular breeding record had a 76 per cent calf crop. The cows received green feed in addition to the sprouted oats. The author concluded that the feeding of sprouted oats was of little value as a treatment for sterility in cows.

Gullickson and co-workers (87) in a study using 15 cows fed a ration totally devoid of vitamin E reported that the sexual development was normal in every case and only two of the heifers required more than one service.

Amino acids

The value of proteins for successful reproductions has been definitely indicated by Guilbert (95) in which he discovered that protein deficiency causes a cessation of ovulation in the female. Cunningham and Hopkirk (91) reported that excessive protein of 65 to 82 per cent in the ration caused some sterility in female rats but not to as great an extent as with males.

Minerals

According to Guilbert (95) phosphorus is essential for reproduction. Quinlan and Roux (7) also reported that in areas where the soil was low in phosphorus that the fertility of the cattle was low. These authors were of the opinion that forced high milk demands are reflected in the genital tract resulting in the failure to show the physiological symptoms of estrum.

Manganese deficiency and its relation to female conception rates have been reported by Lardy and associates (52), Johnson (47), and Boyer et al. (51). The latter workers using rats reported that the females on low manganese diets showed a delay of twice as long in the opening of the vaginal orifice as did the normal animals.

The ill effects of Fowlers solution which contains arsenic upon rabbits was discovered by Roberts and Dawson (49). The services required for conception increased from 1.08 to 1.78. The litter size was also reduced and there was a much higher mortality of the young.

The destructive effect of potassium iodide upon the vitamin C of the blood has been reported by Brown and associates (63). They stated, "that it might be possible for this mineral to reduce the breeding efficiency of a herd."

Summary of Review of Literature

Reproduction in the Male

The factor affecting libido in bulls has not received very much study. It appears that a ration which improves vitamin C snythesis or the injection of ascorbic acid will aid in maintaining sex drive. The data showing the effect of feeding green feeds and sources of vitamin E on libido in the male is contradictory. The feeding of manganese deficient diets to rats resulted in sterility in the males. The feeding of arsenic depressed libido in rabbits. A marked lack of thyroid activity is associated with impaired sex interest. Exercise may increase libido in the bull.

The quality of semen appears to be lower during the summer months. Exercise and the frequency of service may influence semen quality. The age of the sire does not appear to have any great affect on semen quality, until the bull has reached seven years of age, after this age semen quality tends to be lower.

Rations appear to have some influence on semen quality. Factors which improve ascorbic acid metabolism may improve semen quality. This vitamin does not augment the effect of gonadotrophic hormones. Marked vitamin A deficiency results in an increase in the number of services per conception. The experimental work conducted in the United States indicate that the feeding of wheat germ oil as a source of vitamin E is of no value in maintaining semen quality. Recent work indicates that the feeding of animal proteins as supplement to bull rations increases semen quality. Manganese is the only mineral element that has been studied to any extent in relation to semen quality. Sterility was produced on a very low manganese ration. Farm feeds contain more manganese than were used in these experiments.

The feeding of chlorobutanol and succinyl sulfathiazole to bulls increased plasma ascorbic acid values and consequently may be of value in the treatment of sterility.

Hormones have been used in an attempt to increase semen quality. The evidence indicates that the anterior pituitary gland is necessary for spermatogenesis. The thyroid gland has no effect in this respect.

Several investigators have studied the preservation of semen. Most workers are of the opinion that a storage temperature of 5° C. was optimum. Values for good quality of semen varied somewhat, but it appears that the initial motility should be 45 per cent or more.

Reproduction in the Female

Two studies have been made on the factors affecting libido in the female; one with the bovine and one with the rat. The use of manganese and the ergot of rye appeared to have some value in increasing libido in the female.

Studies on the factors influencing conception in the female have not been as extensive as those with the fertility of sires. This is due to the fact that reproductive problems of the female bovine affect only one individual, whereas infertility in the sire affects the entire herd. Female bovines do not lose their reproductive efficiency as early in life as the males. No difference is noticeable up to nine years of age. Heifers require more services for the first conception than succeeding ones. Lethal factors which decrease fertility may be inherited.

Vitamin C appears to be closely related to conception. The vitamin C content of the corpus luteum is high early in gestation period. The administration of vitamin C along with gonadatropic hormones in guinea pigs resulted in an increase in the size of the ovaries.

Vitamin A does not appear to be a contributing factor in reproductive failure in the female bovine under farm conditions. Vitamin E is not a factor in bovine fertility. Phosphorous and manganese deficiencies may cause breeding difficulties. On the other hand the feeding of excessive amounts of arsenic and potassium iodide caused reduced fertility.

OBJECT

The objectives of this experiment were to determine: (a) the effect of increasing the blood plasma ascorbic acid by the use of chlorobutanol or some other stimulating effects of the drug to improve the libido of the male bovine; (b) to measure the improvement of the quality of the spermatozoa of male bovine with poor breeding histories; and (c) to study the influence of the drug on the conception rates of the "hard to settle" female bovine.

EXPERIMENTAL PROCEDURE

Animals Used

The bulls and cows used in this experiment had histories of having breeding difficulties. Therefore, they could not all be housed at the College Experimental barns because of lack of room and labor to take care of them. Many of the animals were left on the farms of the owners and data were acquired with their cooperation.

A few of the animals in the experiment were from the main breeding herds of the Dairy Departments of Michigan State College and Rutgers University.

Feeding Procedure

The animals were fed the chlerobutanol in the grain ration. The entire quantity for a one-day dosage was fed in the grain at one time. The basic dosages were fed at the rates indicated by Bortree et al. (16). Variations from these amounts were made for various animals largely on a weight basis. The chlorobutanol was supplied to the cooperators by the

Michigan Agricultural Experiment Station. Instructions for keeping the records were also furnished.

Bleeding Procedure

The animals were bled from time to time during the experiment when it was convenient to bring the blood samples to the laboratory within two or three hours time. Because of war time restrictions of travel, it was impossible to bleed many of the animals as often as desirable.

In all cases the samples were drawn in 25 ml. tubes. The anticoagulant used was five or six drops of a 20 per cent solution of potassium oxalate. The blood was mixed with the anticoagulant by turning the stoppered tubes end over end in a slow manner. They were then placed on ice and protected from the light while being transported to the laboratory.

Determination of Plasma Ascorbic Acid

The procedure used for the analysis was that described by Mindlin and Butler (19) with certain slight modifications for the micromethod. The usual practice of adding a five per cent solution of potassium cyanide to the blood was omitted as several workers had reported that it gave no added protection to the ascorbic acid. The samples were centrifuged as soon as they reached the laboratory. The filtrate was prepared by pipetting two ml. of the plasma into a test tube containing two ml. of the distilled water. To this was added four ml. of a five per cent solution of metaphosphoric acid and mixed by shaking gently. The mixture was filtered through a number two Whatman filter paper and the filtrate collected. The next step was altered somewhat from the usual micromethod as the only instrument available was for the micromethod. Only one ml. of the filtrate was added to one ml. of the dye and the amount of dye reduced was measured colorimetrically. To remove the error of a visually undectable turbidity of the filtrates the samples were reduced further after making the reading for the sample. Corrections were then made for the final reading. In calculating the results the original formula was used and the results multiplied by four to compensate for using only one ml. of filtrate.

The indophenol-acetate solution was made up as described by Mindlin and Butler (19) except it was diluted so that the blank analysis gave a galvanometer reading of 70. This weaker dye solution gave a wider range between the blank reading and the sample reading and reduced the error in the results. Duplicate readings were made in a majority of the determinations on each filtrate and an average of the two used in each calculation. The K value which was used in the calculations was checked at various intervals by the use of standard solutions made up in 2.5 per cent metaphosphoric acid solution with crystalline ascorbic acid.

Determination of Semen Ascorbic Acid

One ml. of semen was diluted with nine ml. of distilled water. Two ml. of the diluted semen was used, and the determinations for ascorbic acid proceeded from this point just the same as for blood plasma. The final value was always 10 times greater in mg. per cent than for blood plasma.

Semen Collection - Spermatozoa Counts and Progressive Motility Evaluations

All samples of semen were collected by using an artificial vagina. The semen was collected in a pyrex centrifuge tube. Immediately after collection the semen was placed in a receptacle containing water at a temperature from 95 to 100° F. Upon arriving at the laboratory one ml. was taken fro ascorbic acid determination and another small quantity was used for making smears and for motility studies.

The remainder of the semen was kept in a tightly stoppered centrifuge tube. To avoid temperature shock due to too rapid cooling the following procedure was used. The tube was wrapped with paper toweling as insulation and then placed in a large pyrex test tube. This tube was placed in a beaker of water at 55 to 60° F. The beaker was then set in a refrigerator at 40° F. Within the insulated cnetrifuge tube the semen cooled slowly at the rate of approximately 5° C. every 10 minutes until the storage temperature of 40° F. was reached. At 24 hour intervals for three successive days small samples of semen were removed from the original sample by pipette and studied for motility. After removing the sample and placing it in a small test tube, the cold diluent which was at the same temperature as the semen was added. Then the diluted semen was warmed rapidly to 95° F. and motility values made immediately. Spermatozoa counts were made at various intervals by using the hemocytometer and following the red blood countprocedure.

Early in the study the motility estimates were made without dilution. A drop of semen was placed on a slide, covered with a cover slide and values established at 450 x magnification. A majority of the determinations were done by diluting with a nutrient diluent described by Willett et al. (81).

During the early part of the experiment motility values were established in four groups as -, --, ---, and ----. The first group represented values from 20 to 40 per cent, the second 40 to 60 per cent, the third 60 to 75 per cent and the fourth over 75 per cent. Only original motility was recorded at that time. Later in the experiment all values that were above five per cent were recorded to the nearest five per cent. This figure was arrived at by evaluating motility at five different areas on the slide and averaging them and placing the value at the closest five per cent.

Evaluating the Degree of Libido

The procedure used was to note the length of time in minutes or fractions of a minute that elapsed from the time the male was stood at a distance of two or three feet behind the female and the completion of the service to the cow.

Semen Smears

Occasional smears were made of the fresh semen to be used for morphological studies. A small drop of semen was placed near the end of a glass slide. A second slide was used to draw the droplet evenly across the glass by contacting the drop with the end of the second glass. After the semen was dried it was set with alcohol and then stained by using a three per cent rose bengal solution.

Measuring the Improvement in Fertility

Breeding performance of a sire was measured by the number of services per conception. To accurately reflect this basis no cow was considered pregnant until she was pronounced safely with calf by a veterinarian or had actually delivered a calf.

RESULTS

Chlorobutanol Administration to Sires

Libido

During a three year period fifteen slow breeding sires were studied to determine the effect of the oral administration of chlorobutanol upon libido. The detailed results with each sire will be found in the Appendix.

The rate of feeding had to be kept at the five gram level for most sires. Only one large Brown Swiss bull could be kept at the ten gram feeding level without manifesting muscle incoordination due to the anaesthetic effect of the drug. It appeared that many of the sires were affected by the drug although they did not lose their muscle coordination. This effect was one of drowsiness. Most owners reported that their libido was much better a week after the feeding of chlorobutanol was discontinued than while the feeding was being done.

Only one sire had any ill effects from eating the drug. In this one case the individual developed a foaming at the mouth when consuming only five grams of the drug per day with the ration.

Recovery of libido was attained in 76.6 per cent of the cases. The time of service was reduced from an average of forty-five to three and seven tenths minutes per bull. The results also indicate that a feeding period of 40 days must be anticipated with a total consumption of one-half pound of the drug.

Breed and Libido Improvement

Whether size was a factor in the speed of recovery appeared as an important part of this study. It was discovered that most bulls could not be fed consistently more than five to seven grams of chlorobutanol without losing their muscle tone. Therefore, it may be assumed that a heavy Holstein bull may require more days of feeding to get the same results. Table II presents the data according to breed. The data for the Guernsey bulls cannot be considered representative since there were only two animals in this group. Furthermore, one of the sires of this breed was 14 years old and was fed in excess of the normal period of time in attempting to get a response.

A comparison of the Jersey group and the Holstein group shows that the Holsteins were twice as large in weight as the Jerseys and that it took an average of 14.7 more days with 41.1 per cent more chlorobutanol to get the same results. It was interesting to note that there was very little difference in the age of the two groups and that they both had an average return to normal libido of 75 per cent.

The Holstein group made more recovery when one consideres the time required for service; however, they were as slow originally as the Jerseys.

Age and Libido Improvement

To determine if the factor of difference in age would have any effect on the degree of recovery the data were assembled in two groups. Eight sires two years old or younger were placed in one group and seven sires three years old or over were placed in another. The data are presented in Table III.

The older group of sires with an average age of 6.6 years were twice as heavy as the younger group and consumed dosages on a daily basis one-third larger than the younger group. The older group took only two more days to recover than the younger group. The main difference between the two groups was the percentage that returned to normal. Only 56.2 per cent of the older group recovered compared to 94 per cent of the younger group. Age of the sires was the greatest single factor in determining the response to the drug.

TABLE I. EFFECT OF FEEDING CHLOROBUTANOL ON THE LIBIDO OF DAIRY BULLS

Name of bull	Breed	Age yrs.	Est. Weight lbs.	Chlorobutanol Feeding: Daily Dose gms.	Chlorobutanol Feeding: Days Fed	Chlorobutanol Feeding: Total Amount Fed gms	Serving Time: Before Treatment min.	Serving Time: After Treatment min.	Normal Libido Recovered	Remarks
Patty	Jersey	2	900	5	44	220	10-15	(5 sec.)	yes	Bull exceptionally fast. Two men required to hold him back
Pheiffer	Swiss	7	1900	10	23	230	60 or more	5	yes	
Green	Holstein	(18 mo.)	1400	5	70	350	15	3	yes	
Butch	Guernsey	(16 mo.)	750	10 5	7) 44)	290	30	1	yes	Produced incoordination
Spotts	Jersey	4	1100	7	34	238	45	2	yes	Normal
Inka	Holstein	6	2500	10 0 5	5) 2) 31)	205	60	5	yes	10 gms. produced incoordination Bull went completely out of service 22 days after feeding was discontinued.
Royal	Jersey	(14 mo.)	700	5	31	155	1-45	15	partially	There was definite recovery although not complete.
Actor	Jersey	6	1200	5-7	13	71	refused	refused	no	Sedative effect very noticeable
Reichle	Holstein	(15 mo.)	1000	5-7	30	162	40	1	yes	Complete recovery after sedative effect disappeared
Flash	Jersey	6	1200	5	57	285	30-60	5	yes	
Gilna	Jersey	(14 mo.)	750	5	21	105	45-60	2	yes	
Segis Boy	Holstein	3	2400	5	63	315	30-60	refused	no	Refused to eat 10 gm. Foamed at mouth with 5 gm.
Argyll	Guernsey	14	1500	5-10	73	475	refused	refused	no	1 shock treatment of 30 gm. daily for 263 da.
Royal "X"	Jersey	(14 mo.)	500	5	40	200	90	5	yes	More active after treatment
Heir	Jersey	(16 mo.)	700	5	40	200	60	5	yes	Brought back to regular service

TABLE II. RELATION OF BREED TO LIBIDO IMPROVEMENT

Name of bull	Age yrs.	Estimated Weight lbs.	Daily Dose gms	Days Fed	Total Amount Fed	Serving Time: Before Treatment min.	Serving Time: After Treatment min.	Normal Libido Recovered
					(Jerseys)			
Patty	2 yr.	900	5	44	220	10-15	(5 sec.)	yes
Spotts	4 yr.	1100	7	34	238	45	2	yes
Royal	(14 mo.)	700	5	31	155	no service	15	partially
Actor	6	1200	5-7	13	71	refused	refused	no
Flash	6	1200	5	57	285	30-60	5	yes
Gilna	(14 mo.)	750	5	21	105	45-60	2	yes
Royal "X"	(14 mo.)	500	5	40	200	90	5	yes
Heir	(16 mo.)	700	5	40	200	60 or more	5	yes
Av.	2.85 yr.	881	5.4	35	184	47.4	4.9	75 per cent
					(Holsteins)			
Grear	(18 mo.)	1400	5	70	350	15	3	yes
Inka	6	2500	5-10	36	205	60	5	"
Reichle	(15 mo.)	1000	5-7	30	162	40	1	yes
Segis Boy	3	2400	5	63	315	30-60	refused	no
Av.	2.77 yr.	1825	5.9	49.7	258	38	3	75 per cent
					(Guernsey)			
Butch	(16 mo.)	750	5-10	51	290	30	1	yes
Argyll	(14 mo.)	1500	5-10	73	475	refused	refused	no
Av.	7.65 yr.	1125	5.5	62	382	30	1	50 per cent

TABLE III. RELATION OF AGE TO LIBIDO IMPROVEMENT

Breed	Age	Estimated Weight	Daily Dose	Days Fed	Total Amount Fed	Serving Time Before Treatment	Serving Time After Treatment	Normal Libido Recovered
	yrs.	lbs.	gms.		gms.	min.	min.	
Two Year Old or Under								
Jersey	2	900	5	44	200	10-15	5	yes
Holstein	(18 mo.)	1400	5	70	350	15	3	yes
Guernsey	(16 mo.)	750	5-10	51	290	30	1	yes
Jersey	(14 mo.)	700	5	31	155	15-45	15	partially
Holstein	(15 mo.)	1000	5-7	30	162	40	1	yes
Jersey	(14 mo.)	750	5	21	105	45-60	2	yes
Jersey	(14 mo.)	500	5	40	200	90	5	yes
Jersey	(16 mo.)	700	5	40	200	60	5	yes
	Av. 1.4 yr.	875	5.2	41.1	210	41	4.6	94 per cent
Three Year Old or Over								
Brown Swiss	7	1900	10	23	230	60	5	yes
Jersey	4	1100	7	34	238	45	2	yes
Holstein	6	2500	5-10	38	205	60	5	yes
Jersey	6	1200	5-7	13	71	refused	refused	no
Jersey	6	1200	5	57	285	30-60	5	yes
Holstein	3	2400	5	63	315	30-60	refused	no
Guernsey	14	1500	5-10	73	475	refused	refused	no
	Av. 6.6 yr.	1686	6.9	43	260	52	4.2	56.2 per cent

Semen Quality

Eleven sires with poor breeding histories were used in this study. Seven of these sires were kept on the owner's farms, since the bulls were needed for service. The data were secured from 10 separate experimental periods. Two sires were studied for response to chlorobutanol in two separate trials. All of the sires used had low conception rates before starting on the experiment. Conception rates were determined on the semen of a majority of the sires used in this study, in order to measure the effect of chlorobutanol feeding on sperm fertility.

"Patty" bull:

This 900 pound two-year-old Jersey sire was fed chlorobutanol as a treatment for sterility. The breeding history during the seven months prior to the feeding of this drug was 5.33 services per conception (32 services and 6 conceptions). During 43 days following the beginning of the treatment, only 1.50 services were required per conception (6 services and 4 conceptions). The effect on blood plasma ascorbic acid and sperm motility are shown in Table VII of the Appendix. The plasma ascorbic acid was 0.22 mg. per 100 ml. on February 28, and gradually increased following the administration of 5 gm. of chlorobutanol daily until a peak value of 0.51 mg. per 100 ml. was observed on March 22. The quality of the semen also improved as was indicated by increased sperm motility. Chlorobutanol was discontinued on April 12. There was a marked drop in plasma ascorbic acid but the sperm motility increased to 75 per cent where it remained for the next five months.

This sire was purchased by the Michigan Agricultural Experiment Station in November, 1941 and placed on a long time experiment. In Mauch, 1942 the ascorbic acid plasma values had dropped to 0.10 mg. per cent. This low value indicated that this sire was subject to ascorbic acid deficiency. It also appeared that the original motility may not be the best measure of the fertility of the sperm. To study this problem further it was decided to make motility determinations at intervals of 24-hour periods for three days. The ascorbic acid content of the blood plasma and semen and the pH. volume and motility of the semen for one year are tabulated in Table XVIII of the Appendix.

During the second chlorobutanol feeding period, 75 days were required to bring the semen of this sire back to normal. There were no experimental cows available for service at this time. The sperm count returned to a normal level in a period of three weeks time after the beginning of chlorobutanol feeding. Almost ten weeks, however, were required to obtain normal liveability of the sperm.

A study was also made of the morphology of the sperm in order to determine the possibility of other casuative factors contributing to poor fertility in this bull. The results are shown in Table IX in the Appendix. At the beginning of the second trial there were 76 per cent abnormal sperms and a majority of these had coiled tails. After a period of four and one-half months of chlorobutanol treatment the abnormal sperms had decreased to 21 per cent. These abnormal sperm had coiled tails. Two and one-half months after feeding of chlorobutanol had been discontinued the per cent of abnormal sperms had again increased to 43 per cent.

Sire "1645":

This sire was a 1500-pound three-year old Holstein-Friesian bull which had a poor breeding history. The number of services per conception for the

11 months prior to chlorobutanol treatment was 3.8. Data showing the effect of chlorobutanol on plasma and semen ascorbic acid and germ motility are shown in Table X of the Appendix.

This sire started with a blood plasma ascorbic acid value of 0.28 mg. per cent which is considered normal. The motility of the semen, however, was very low. A maximum ascorbic acid level in the blood was reached within three weeks time. In three months time the motility of the semen had returned to a normal value. Five months after the feeding of chlorobutanol had been discontinued the semen ascorbic acid values were declining and the number of services per conception had increased to 11. Feeding of chlorobutanol was again started. In two months time both the ascorbic acid values and motility had returned to normal.

In Table XI of the Appendix the breeding efficiency of this sire is evaluated. During the first five month feeding period, 13 services resulted in five conceptions or 2.60 services per conception, compared to 42 services and 11 conceptions or 3.80 services per conception prior to chlorobutanol feeding.

After feeding of chlorobutanol had been discontinued for five and one-half months, the semen values had decreased rapidly. Feeding of chlorobutanol was again started.

During the preceding three month period prior to the start of the second trial, eleven services resulted in only one conception. After feeding 1170 grams of chlorobutanol over a four months period in the second trial seven services resulted in three conceptions or 2.33 services per conception.

<u>Sire "72-A":</u>

Sire 72-A was a Holstein-Friesian bull, five years of age, weighing 2200 pounds. The effect of feeding chlorobutanol on the ascorbic acid content of the plasma and semen, and sperm motility is shown in Table XII of the Appendix. This bull received 1080 gm. of chlorobutanol over a period of 149 days. Although Sire 72-A weighed 2200 pounds, it was only able to tolerate the sedative action of 10 grams of chlorobutanol per day for 38 days. Five grams of chlorobutanol per day was not sufficient to maintain a plasma ascorbic acid level at a sufficiently high plane to produce viable semen. An average dosage equivalent to about seven grams per day appeared satisfactory.

Although the blood plasma level reached a peak of 0.5 mg. per cent and the motility reached normal values during the 149 day feeding period, it was never possible to get the ascorbic acid level of the semen up to 5 mg. per cent.

During the three months prior to chlorobutanol administration the breeding history showed 5.66 services per conception (17 services and 3 conceptions), while during the first four months of treatment the number of services per conception was 2.60 (13 services and 5 conceptions). This data is presented in Table XIII of the Appendix.

Sire "1601":

This sire was a two-year-old Holstein-Friesian bull weighing 1600 pounds. It had a normal amount of ascorbic acid in the semen prior to the feeding of the chlorobutanol. Even though this situation existed the spermatozoa seemed to be of low fecundity as the sire required more services per conception than normal. Data with reference to the blood and semen ascorbic values and motility of the sperm is found in Table XIV of the Appendix.

Table XV in the Appendix presents data on the number of services per conception. During the month prior to treatment the ratio of services to conception was 3.00 (6 services and 2 conceptions). The feeding of chlorobutanol for one month reduced this ratio to 1.36 (13 services and 11 conceptions) over a period of four months.

Sire "Shorthorn":

This sire was a five-year-old bull weighing 2100 pounds which was sterile at the time the feeding of chlorobutanol was started. All cows bred to this sire for a period of several months had failed to conceive. The bull was brought to the Dairy Experiment Station barn for study. The semen was of poor quality as evidenced by the short time liveability of the sperm. Data in Table XVI of the Appendix show that after chlorobutanol had been fed for some time, the semen did not show any marked change in ascorbic acid content. The sperm, however, showed marked improvement in liveability. No opportunity presented itself to test the fertility of this sire before it had been disposed of to make room for other animals.

Table XVII in the Appendix presents data on the per cent of abnormal spermatozoa in the semen of this bull. The semen had 13 per cent abnormal sperm a month after chlorobutanol feeding had started and nien per cent when feeding was discontinued. Two months after the feeding was discontinued the abnormal sperms were back to 12 per cent. At all times a majority of the abnormal sperm had bent bodies and coiled tails.

Sire "Wells":

The "Wells" bull was a Jersey three-year-old and weighed 1000 pounds. During the three months prior to treatment the number of services per conception for this animal was 5.0 (10 services and 2 conceptions). During the two months after starting chlorobutanol, the conception rate was 1.5

(3 services and 2 conceptions). See Table XIX in the Appendix.

The plasma ascorbic acid values for plasma and semen were not changed significantly during the first month of chlorobutanol administration. Data were not collected on this animal from June 6 to July 1. Chlorobutanol feeding was started June 26. The plasma ascorbic acid values became markedly higher. Semen ascorbic acid values did not show an increase until July 15. Chlorobutanol was discontinued August 17. The plasma and semen ascorbic acid values remained within the normal range until the bull was sold. There was an improvement in sperm motility of the fresh sample and at 24 hours associated with increased semen ascorbic acid as shown in Table XVIII of the Appendix.

Sire "D-520":

Sire D-520 was a four-year old bull which weighed 2000 pounds. The ascorbic acid content of the blood plasma and the semen data are presented in Table XXI of the Appendix. This sire had been out of service for several months prior to the time this study was begun. The spermatozoa were all non-motile. Heavy doses of chlorobutanol were administered orally by capsule in order to stimulate the rapid synthesis of ascorbic acid. Chlorobutanol feeding was started June 20. The feeding of 170 grams of chlorobutanol over a 9-day period resulted in muscular incoordination. Although the blood plasma ascorbic acid values attained in four weeks time were as high as those in the other sires used in the experiment it was impossible to bring the semen values into a normal range. Most of the samples were seminal fluid without sperm. Considerable amount of cellular debris and crystals were noticed in the seminal fluid from time to time and on one occasion the fluid was dark brown in color.

The feeding of 5,000,000 I.U. of vitamin A in the form of shark liver oil had no effect on the plasma and semen ascorbic acid values.

The pH values on most samples of seminal fluid or semen were higher than found on normal samples of semen.

Sire "Thorn":

This bull was a seven-year-old Jersey weighing 1100 pounds. Data on the blood and semen ascorbic acid values and sperm motility are found in Table XXII of the Appendix. This sire was able to tolerate a 10-gram dose of chlorobutanol for 23 consecutive days before developing muscular incoordination. A total of 810 grams of chlorobutanol was fed over a 61 day period.

The problem with this sire was to improve the fecundity of the sperm. When the sire was placed on the trial the semen was normal as to sperm motility for fresh samples, at 24-hour and 48-hours of age. Although this was true the conception rate for the three months period prior to the experiment was 4.33 (13 services and 3 conceptions). During the three month period following the 61 day feeding trial the conception rate was 1.40 (7 services and 5 conceptions). Data for conception rates are found in Table XXII of the Appendix.

Sire "Ladd":

Results of feeding chlorobutanol to sire "Ladd" a six-year-old Jersey bull weighing 810 pounds are tabulated in Table XXIV of the Appendix. The feeding of 425 grams of chlorobutanol over a period of 85 days resulted in an increase in per cent of motility over the entire 72 hours evaluation period during the first two months and then declined during the rest of the chlorobutanol feeding period.

Data in Table XXV of the Appendix show that the breeding efficiency of this sire was not improved to any extent. Before treatment the number of services per conception was 9.00 (9 services and 1 conception). After treatment it was 7.5 services per conception (15 services 2 conceptions). These negative results were probably due to the presence of 30 per cent abnormal sperm and considerable amount of cellular debris in the semen.

Sire "Rex":

Rex, a five-year-old Guernsey sire weighing 1400 pounds was treated on the owner's farm. The only record that could be obtained during the war time travel restrictions was improvement in breeding efficiency. Chlorobutanol was fed at the rate of five grams per day for 60 days. The results on breeding efficiency are as follows:

Period	Treatment	Services	Conception	Services per Conception
3 months previous	None	10	3	3.33
3 months following	60 days	8	6	1.33

Sire "Maxim":

This seven-year-old Guernsey bull weighing 1500 pounds was fed chlorobutanol at the rate of five grams per day for 60 days. This sire was also treated at the owner's farm. The results on breeding efficiency are as follows:

Period	Treatment	Services	Conception	Services per Conception
3 months previous	None	13	5	2.60
3 months following	60 days	10	7	1.45

Measuring the Improvement in Fertility

Services per conception for the ten experimental periods using nine sires are summarized in Table IV. These sires required an average of 5.21 (2.6 to 11.0) services for each conception prior to the oral administration of chlorobutanol. A sire with 2.60 services per conception might be considered a border-line case of low breeding efficiency.

Table IV. SUMMARY OF THE USE OF CHLOROBUTANOL ON THE BREEDING EFFICIENCY OF SIRES.

Name of Sire	Days Fed Chlorobutanol	Grams of Chloro-butanol Fed	Services per Conception Before Feeding	Period Before (Weeks)	Services Per Conception After Feeding	Period After (Weeks)
1. Patty	42	120	5.33	28	1.50	6
2. "	102	490	not used	-	-	-
3. 1645	55	275	3.80	44	2.60	20
4. 1645 (2d period	158	1170	11.00	12	2.33	16
5. 72-A	149	1080	5.66	12	2.60	16
6. 1601	28	190	3.00	4	1.36	4
7. Wells	60	390	5.00	12	1.50	8
8. Thorne	61	810	4.33	12	1.40	12
9. Ladd	85	425	9.00	12	7.50	12
10. Rex	60	300	3.33	12	1.33	12
11. Maxim	60	300	2.60	12	1.45	12
Total	860	5840	52.05	160	23.57	118
Av.	78.1	530.9	5.21*	16.0*	2.36*	11.8*

*Average calculated only on the trials where the sires were used.

After chlorobutanol had been fed the average number of services per conception was reduced to 2.36 (1.33 to 7.50). The sire having 7.50 services per conception did not respond to chlorobutanol treatment. On a group basis 81 per cent of the sires improved in fertility of the sperm after chlorobutanol was fed. The average number of days that chlorobutanol was fed was 78.1 (42-158) and the average number of grams of the drug that was fed was

530.9 (190-1170) grams. No ill effects were observed from feeding the chlorobutanol even for the longest period of time.

Nature of Improvement in Fertility

Therapy for poor fertility in sires by the use of oral administration of chlorobutanol developed with a definite pattern. Data covering this phase of the project is found in Table V.

The improvement was first noticed in increased blood plasma ascorbic acid values. The average days required to reach maximum levels of plasma ascorbic acid for the group of sires studied was 38.7 days. The range was from 20 to 60 days.

Fresh semen samples returned to a 75 per cent progressive motility in 60.4 days for the group. A wide variation also occurred in this category. The most rapid response occurred in fifteen days compared to 128 days for the bull showing slow recovery.

TABLE V. LENGTH OF TIME REQUIRED TO OBTAIN IMPROVEMENT IN BLOOD AND SEMEN VALUES AFTER FEEDING CHLOROBUTANOL

Name of Sire	Age Yrs.	Days to Obtain			Improvement in Conception Rate
		Maximum Blood Ascorbic Values	Maximum Semen Ascorbic Values	Fresh Sample 75 % Motility Days	
Patty	2	20	27	54	Yes
Patty (2d)	3	60	32	60	Not used
1645	3	24	115	80	Yes
1645 (2d)	4	23	127	63	Yes
72-A	5	24	143	128	Yes
1601	2	36	36	36	Yes
Shorthorn	5	60	67	60	Not used
Wells	3	60	75	60	Yes
D-520	4	32	121	Failed	No
Thorne	7	48	55	15	Yes
Ladd	6	-	-	48	No
Rex	5	-	-	-	Yes
Maxim	7	-	-	-	Yes
Total	56	387	798	60.4	Yes - 81 %
Av*	4.3	38.7	79.8	60.4	

*Average calculated only on number of sires for which there was data in each column.

Maximum ascorbic acid values developed much more slowly in semen than in the blood. The average length of time required to reach the highest values in the semen was 79.8 days. Here again, the variation in time was from 27 to 143 days. The sire showing the slowest recovery in per cent of sperm motility required the highest number of days to attain the maximum amount of plasma ascorbic acid in the semen. Furthermore, the sire that reached the maximum blood values in the shortest length of time also attained the maximum semen values in the shortest length of time.

Administration of Large Doses of Chlorobutanol

Large does of chlorobutanol were administered in an attempt to reduce the time required to bring about recovery of fertility. Sires studied as reported in Tables VIII, XII, XVI, XVIII and XXI of the Appendix received doses of more than five grams per day for various lengths of time. Individual sires showed considerable variation in their ability to tolerate massive dosages of chlorobutanol.

The Patty bull (Table VIII in the Appendix) withstood 20 grams per day for nine days without developing muscular incoordination. This period was followed by four 30 gram doses in capsules over a period of eight days. At the end of this time pronounced incoordination of the muscles developed.

With Sire 72-A (Appendix Table XII) ten gram doses were fed for 38 days, then five grams for 65 days. Following this period this sire was again fed a ten gram dose and in nine days time developed incoordination of the legs.

The Shorthorn bull (Table XVI in the Appendix) was fed a ten gram dose for 21 days before evidence of muscle incoordination developed. The Wells bull withstood ten grams per day for ten days, however, the heavy dose equivalent to 15 grams per day fed at the rate of 30 grams every other day produced pronounced muscle incoordination in eight days time. With the D-520 bull (Appendix Table XXI) 20 and 30 gram doses fed by capsule produced a positive incoordination in nine and eight days, respectively.

Blood plasma ascorbic acid values increased more rapidly following the massive dose treatment than when small doses were administered. None of the sires exhibited an ill effect after the large doses were discontinued and normal doses of chlorobutanol were again fed.

Morphology of the Sperm

Four sires were studied with reference to the morphology of the spermatozoa during a part or the entire period of the experiment.

Table IX of the Appendix shows the history of the Patty bull. This sire improved rapidly in the appearance of the spermatozoa. After the therapy was discontinued the number of abnormal spermatozoa again increased. In four and one-half months of treatment the abnormal spermatozoa dropped from 76 per cent to 21 per cent and then in two and one-half months without treatment returned to 42 per cent.

Tables XVII and XX of the Appendix present the data on the "Shorthorn" and "Wells" bulls. In both of these animals the per cent of abnormal spermatozoa was not above the average for high quality semen. The sperms of neither one of these two bulls showed any improvement from a morphological standpoint. The Ladd bull ahd 30 per cent of abnormal sperm after feeding had been in progress for a period of a month. This may explain in part the reason why this sire did not improve with reference to fertility of the sperms.

Chlorobutanol Administration to Cows

Scope of Study

In this part of the experiment 32 cows with breeding difficulties in four different herds were studied. Two of the herds were privately owned. One was Michigan State College herd and the other the Rutger's University herd. All five breeds of dairy cattle are represented in this study. The results are tabulated in Tables XXVI to XXX inclusive of the Appendix and summarized in Table VI.

All of the 32 cows had been examined by veterinarians and pronounced normal from a clinical standpoint with reference to their reproductive organs. Fifteen of the 32 cows or 46.9 per cent had been bred to two or more sires, which tended to eliminate the possibility of the lack of fertility caused by the sire rather than the female under study.

Effect of Treatment on Milk Production

In Table XXVI of the Appendix data are presented showing the effect of normal therapy and shock therapy (massive doses) on the rate of milk flow when lactating female bovines were treated. When five gram doses were used no appreciable decline in milk flow was experienced other than the normal decrease due to stage of lactation. Prior to the treatment on five milking cows the average production per day was 33.5 pounds of milk. During treatment the production averaged 31.8 pounds of milk. Shock therapy caused a sharp decline in milk flow amounting to slightly over a 50 per cent decrease.

TABLE VI. SUMMARY OF EFFECT OF FEEDING CHLOROBUTANOL TO "HARD TO SETTLE" COWS

Cows	Breed	Weight	Services Before Treatment	Dosage Per Day	Services to Conception After Treatment	Days Fed Chlorobutanol
		Lbs.	No.	Grams	No.	
Surprise	Jersey	850	6	5	1	21
Joy	"	600	9	5-20-5	6	42
Maid	"	800	6	5	1	21
Jewel	"	850	14	5-20-5	7	42
Bramble	"	850	5	5	1	21
Marcia	"	900	8	5-20-5	4	42
Vera	"	900	5	5	1	21
Naidna	"	850	5	5	1	21
Valiant Girl	"	800	8	5-20-5	8	42
652	Br. Swiss	1285	7	5	1	39
640	"	1175	4	5	1	21
621	"	1377	3	5	2	39
J-4	Jersey	735	4	5	1	38
J-5	"	766	6	5	1	38
393-X	"	1130	4	5	1	24
J-2	"	908	5	5	1	38
J-3	"	800	4	5	1	38
A-1	Ayrshire	1285	2	5	2	39
634	Br. Swiss	1308	3	5	2	39
650	"	1250	6	5	2	39
A-27	Holstein	972	3	3	1	16
A-29	"	1080	5	4	1	28
A-31	"	980	3	3	2	36
A-37	"	1075	3	3	1	29
A-39	"	1015	4	3	1	20
66	Jersey	750	4	3	1	65
412	Holstein	1050	3	4	1	29
288	"	1569	3	4-8	4	100
Sadie I	Guernsey	1350	4	5	1	35
Snowball	"	1000	4	5	1	24
Admiral's Clara	"	1100	1	5	6	75
Admiral's Mary	"	1000	4	5	2	45
Total		32360	155		64	1167
Av.		1011	4.84		2.00	36.5

DISCUSSION OF RESULTS

Effect on Libido in Bulls

Response to the feeding of chlorobutanol took place usually within a week to ten days. The average number of days that chlorobutanol was fed was 41.8. The range was from 13 to 70 days. The most satisfactory dose appeared to be about five grams per day. Some sires tolerated heavier doses than others. The "Spotts" bull withstood seven grams per day for 34 days as shown in Table I.

Blood plasma ascorbic acid values increased greatly in sires "Patty" and "Pheifer Boy" as shown in Table XXXI in the Appendix. These data are in agreement with the results of Bortree, Huffman and Duncan (15), (16). Phillips (26) obtained definite response in "slow breeding" bulls by the subcutaneous injection of vitamin C. Chlorobutanol may have produced libido stimulating effect in part at least through increased blood plasma ascorbic acid.

The failure of the 13.4 per cent of the bulls to respond to chlorobutanol may have been due to improper function of some other endocrine gland. Phillips (14) was of the opinion that ascorbic acid has a beneficial effect on the pituitary. Turner (46), Peterson and et al. (43) and Spielman and co-workers (94) associated lack of libido in sires with low thyroid activity.

There was no difference between Holsteins and Jerseys in the rate of improvement in libido as shown in Table II.

The administration of chlorobutanol reduced the average time of service from 45 minutes to three and seven-tenths minutes. Each sire required an average of 233.4 grams of the drug or the equivalent of about one-half pound.

Improvement of libido in slow breeding male bovines was attained in 76.6 per cent of the animals used in the experiment. The loss of libido is not entirely a problem that develops with old sires since 53.3 per cent of the bulls reported as slow breeders in this experiment were under two years of age.

Age of the sire was the greatest factor which influenced the degree of response. With the bulls two years old or younger, there was a 94 per cent recovery. Only one sire, two year old or younger, failed to recover completely. The partial recovery was called one-half recovery in calculating the percentage. In the older group of bulls the average age was 6.6 years. Four of these bulls recovered normal libido, while three bulls failed to recover to any noticeable extent. The average recovery for this group was only 56.2 per cent.

Effect of Feeding Chlorobutanol on Fertility of Bulls

In this study the oral administration of chlorobutanol resulted in improvement in fertility of nine of the eleven sires or an average of 81 per cent as was indicated by the number of services that were required per conception. These results are in close agreement with those of Phillips (26) in which three out of four or 75 per cent of the sires improved in conception rates by the subcutaneous injection of vitamin C.

It was necessary to feed chlorobutanol for a longer period of time in order to improve the fertility of the sperm than to improve the libido. The data show that an average of 78.1 days were required to improve fertility and only 41.8 days to improve libido. The improvement in fertility was indicated by the following results: (a) The blood plasma ascorbic acid value reached a maximum in 38.7 days. (b) The fresh samples of semen showed 75 per cent progressive motility within a mean of 60.4 days. It is interesting to note that ascorbic acid content of the blood of the sires took more days to reach a maximum content than for the motility to reach 75 per cent or more for the fresh semen samples. It took on the average 79.8 days for the semen samples to reach maximum vitamin C values. The 60.4 days required for the semen values to reach 75 per cent progressive motility on the fresh samples after starting chlorobutanol administration is considerably longer than the five weeks of treatment with subcutaneous injections of vitamin C as reported by Phillips and co-workers (26).

The bulls low in fertility as reported by breeders for this study averaged 4.3 years of age. The ages of the two sires that did not respond to treatment as reported in Table V were four and six years, respectively.

The data presented in Table IV show that the bulls which received chlorobutanol were not brought back to the average of 1.56 services per conception as reported by Bowling and associates (1) or 1.52 for bulls under five years as reported by Hilder and co-workers (4). The data in Table VIII show that in six of the ten trials or 60.0 per cent of the sires that were used for breeding had a breeding efficiency of 1.50 services or less per conception after treatment.

All of these sires were fed rations that should have maintained fertility. None of these bulls were in poor flesh due to malnutrition when they were reported as having a poor breeding efficiency. It was observed that the animals usually took on somewhat of an added bloom during the period when chlorobutanol was fed. No apparent detrimental effects were observed from the chlorobutanol feeding up to 158 days. These results are in agreement with the work of Lundquist and Phillips (96) who reported that the feeding of chlorobutanol at the rate of five grams per day had no detrimental effect on the synthesis of the vitamin B-complex. This is of interest because chlorobutanol has a small phenol coefficient.

Chlorobutanol therapy as an aid to correcting abnormal sperms could not be measured since there was only one sire that had a high percentage of abnormal sperm. However, there was an improvement in the morphology of the sperm during the time chlorobutanol was fed in the case of this bull. An interesting observation was that the abnormal sperm with coiled tails in many instances lived longer than the normal sperm. In addition their path of locomotion was reverse to that of morphologically perfect sperms.

Effect on Breeding Efficiency in Cows

In measuring the value of the oral administration of chlorobutanol to "hard to settle" cows, only cows that had a clinically normal reproduction tract were used. The basis for using the chlorobutanol was that Phillips and co-workers (24) had reported that the subcutaneous injection of vitamin C resulted in the improvement of 60 per cent of the cows with breeding difficulties. Ley (30) working with humans showed that ascorbic acid administration aided pregnancy in women by keeping up the ascorbic acid content of the corpus luteum. Davis and Cole (3) reported favorable results from the subcutaneous injection of vitamin C in the breeding performance of mares. Since Bortree et al. (15), (16) had shown that blood plasma levels could be raised measurably by orally administrating chlorobutanol it appeared likely that the use of this compound might be valuable for overcoming shy breeding in the female bovine.

The number of services prior to feeding the chlorobutanol ranged from one to 14. The breeder who started the cow on chlorobutanol after the first service did so because the cow had a history of shy breeding. Twenty of the 32 cows that had an average of 4.55 services prior to treatment conceived on the first service after treatment. The entire group of 32 cows had an average of 4.84 services per conception before treatment. The feeding of the drug was usually started immediately following the cessation of the heat period and continued for at least 21 days. The average feeding period for the 32 head was 36.5 days.

Following chlorobutanol therapy, the 32 cows averaged 2.00 per services per conception. Twenty of the 32 animals or 62.5 conceived on the first service after therapy. The 12 cows which required more than one service, averaged 5.33 services before treatment, and 3.83 services following treatment.

It is likely that some of the 32 cows reported in this study would have eventually conceived without treatment. Asdell and associates (103) using 47 sterile dairy cows reported that the control group eventually had 50 per cent of the cows conceive while those that had been treated with gonadic and gonadotropic hormones only 42.9 per cent conceived. In this experiment all the cows treated conceived, which indicated that the treatment with chlorobutanol improved the breeding efficiency.

Effect of Shock Therapy

As shown in Table VI, there were five cows included in this study that were treated with massive or shock doses. Four cows received 20 grams daily for a period of five days and the fifth cow received eight grams for 22 days. These five cows had an average of five services prior to treatment. All of the cows conceived on the first service except one, following the shock treatment. This cow required only two services.

Five of the 32 cows were fed three grams of chlorobutanol per day instead of five grams. The average number of services before treatment was 3.4. Following treatment all the cows conceived on the first service except one which required two services.

Effect on Milk Production

Milk production was affected only slightly by the normal dosage of three to five grams of chlorobutanol per day as shown in Table XXVIII in the Appendix. Shock or massive doses of 20 grams of chlorobutanol per day reduced milk flow 56.5 per cent. This may have been due to the reduction in appetite associated with the sedative effect of chlorobutanol.

Whether or not the action of the chlorobutanol is a primary or secondary one was undetermined. The ascorbic acid may provide the correct media for the enzyme which breaks down the cell wall of the ovum permitting the sperm to enter.

The return of the treated cows from 4.84 services before treatment to an average of 2.00 services per conception after treatment may be interpreted as returning the cows to a normal condition. This statement is based on the report of Bowling and collaborators (1) who found that the averages of all services for the lifetime of a cow to be 2.02.

SUMMARY AND CONCLUSIONS

1. Fifteen slow breeding bulls were fed chlorobutanol orally. The libido of the bulls improved in 76.6 per cent of the cases. The average time required to serve a cow was reduced from 45 minutes to 3.7 minutes.

2. There was no difference between Jersey and Holstein-Friesian sires in response to chlorobutanol administration from the standpoint of libido.

3. Sires two years or under had an average recovery of 94 per cent compared to 56.2 per cent recovery for a group of sires that averaged 6.6 years of age.

4. Plasma ascorbic acid values showed a peak 38.7 days (average) after starting chlorobutanol feeding. The maximum semen ascorbic acid value, however, was not reached until 179.8 days (average) following chlorobutanol administration.

5. The feeding of chlorobutanol to nine sires with histories of low breeding efficiency which were in service after treatment resulted in the reduction of the number of services per conception from 5.21 to 2.36. This treatment improved the breeding efficiency of 81 per cent of these bulls.

6. In this study an average of 60.4 days was required for the fresh semen to attain 75 per cent or more of progressive motility after starting chlorobutanol feeding.

7. No ill effects were observed from feeding a total of 10 grams of chlorobutanol over a period of 158 days, or an average of five grams per 1000

pounds of body weight per day. Only two sires, however, were able to withstand doses of 20 grams per day over more than a three week period without developing muscle incoordination.

8. Sires used in the improved breeding efficiency and libido studies were fed average daily doses of 6.8 and 6.1 grams of chlorobutanol, respectively. These doses were equal to 4.9 and 5.0 grams per 1000 pounds of live weight, respectively.

9. The most satisfactory daily dosage for feeding periods of 60 to 150 days appears to be five to seven grams.

10. Thirty-two shy breeding cows with an average of 4.84 previous services were used in this investigation. Twenty of these cows conceived on the first service following the feeding of five grams of chlorobutanol per day for about 21 days. Five of the 12 cows which did not conceive on the first service were fed large doses of chlorobutanol (up to 20 grams per day) until they showed incoordination. Four of these five cows conceived on the first service following this treatment while the fifth cow conceived on the second service. Twenty-four of the 32 cows used in this study received an average daily dose of five grams of chlorobutanol per 1000 pounds live weight. Doses of three and four grams per day appeared just as effective as five gram doses. All 32 cows used in this study conceived after treatment with chlorobutanol with an average of two services per conception.

11. Five lactating cows which were fed five grams of chlorobutanol per day declined only five per cent in milk production. Milk production decreased 56.5 per cent in two cows fed 20 grams of chlorobutanol per cow per day.

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APPENDIX

TABLE VII. EFFECT OF FEEDING CHLOROBUTANOL TO THE "PATTY" BULL (JERSEY)

Date	Ascorbic Acid per 100 ml. Blood Plasma mg.	Ascorbic Acid per 100 ml. Semen mg.	Sperm Motility Evaluation Fresh Sample (1)	Chlorobutanol Treatment
2/28/41	0.22	10.47	+	
3/2/41				Started feeding 5 gm. per day
3/7/41	0.28	4.80	+	
3/14/41	0.412	8.83	+ +	
3/22/41	0.510	7.73	+ + +	
3/29/41	0.270	8.89	+ +	
4/4/41	0.397	7.52	+ + +	
4/12/41	0.350	8.77	+ + +	Discontinued chlorobutanol
4/25/41	0.079	7.71	+ + + +	
5/3/41	0.123	5.27	+ + + +	
5/17/41	0.246	5.59	+ + + +	
5/31/41	0.187	6.41	+ + + +	
7/14/41	0.291	6.90	+ + + +	
9/22/41	0.238	8.04	+ + + +	

(1) + = 20-40 per cent motility; + + = 40-60 per cent motility; + + + = 60-75 per cent motility; + + + + = 75 per cent motility.

TABLE VIII. PLASMA ASCORBIC ACID AND SEMEN DATA FOR THE "PATTY" BULL (SECOND TRIAL)

Date	Ascorbic Acid per 100 ml. Blood mg.	Ascorbic Acid per 100 ml. Semen mg.	Sperm Conc. m/cc	Vol. c.c.	Semen pH	Per Cent Motility Fresh	24 hr.	48 hr.	72 hr.
3/27/42	0.100	0.572	-	3.0	-	40	20	0	0
4/3/42	0.200	3.68	-	3.0	-	10	0	0	0
4/17/42	0.244	1.456	-	2.0	-	70	20	0	0
4/21/42	0.190	2.083	-	3.5	7.12	50	0	0	0
4/23/42	0.150	1.766	712	3.0	-	30	10	0	0
4/28/42	0.098	1.953	432	3.0	7.08	40	20	10	10
5/5/42	0.235	4.269	160	3.0	7.30	40	5	0	0
5/7/42	Started feeding 10 gr. chlorobutanol every other day								
5/7/42	0.216	4.685	176	3.5	7.14	40	20	10	0
5/12/42	0.235	5.280	288	4.5	7.40	40	10	0	0
5/19/42	0.350	5.346	260	6.5	7.12	50	20	10	0
5/26/42	0.329	6.565	808	3.0	7.28	60	5	0	0
5/28/42	Discontinued 10 gr. everyother day after feeding 100 gr.								
6/2/42	0.312	6.747	-	5.0	6.90	40	10	0	0
	From 5/29 to 6/6 fed 4 20-gr. doses								
6/9/42	0.236	9.105	872	3.0	6.98	30	15	0	0
	6/9 to 6/17 fed by capsule four 30-gr. doses. In-coordination								
6/18/42	0.360	4.120	-	5.8	7.06	70	20	0	0
6/26/42	Started feeding 10 gr. doses usually every other day								
7/1/42	0.321	3.500	-	4.5	7.40	0	0	0	0
7/7/42	0.573	1.941	-	6.0	6.48	75	10	0	0
7/15/42	0.288	5.869	-	4.0	6.88	65	3	0	0
7/22/42	0.430	4.927	-	5.2	7.02	80	35	15	0
7/28/42	0.494	6.797	-	3.2	6.88	70	50	30	20
7/21/42	0.316	-	-	-	-	70	40	20	5
8/4/42	0.369	7.870	-	5.0	6.86	80	40	40	10
8/10/42	0.371	4.059	-	3.2	-	70	55	35	5
8/17/42	0.373	6.296	-	1.7	6.9	45	20	20	0
8/17/42	Fed 190 gr. since 6/26 - discontinued feeding								
8/24/42	0.419	4.370	-	2.5	7.0	35	35	30	25
8/21/42	0.405	4.531	-	3.0	6.8	70	55	15	0
9/4/42	0.182	5.830	-	4.0	7.0	60	45	15	0
9/12/42	0.252	5.790	-	4.4	-	70	25	1	1
9/22/42	0.323	6.059	-	1.7	-	75	25	15	10
9/20/42	0.319	5.479	-	1.3	-	15	15	10	1
10/15/42	0.340	3.134	-	2.6	-	50	25	20	1
10/20/42	0.362	4.226	-	4.0	-	80	20	10	0
10/27/42	0.272	2.537		1.5	6.9	85	10	0	0
11/3/42	-	2.070	-	1.6	7.12	55	10	0	0
11/10/42	0.403	3.948	-	2.0	7.25	45	35	15	5
11/17/42	0.374	3.655	-	3.2	-	70	60	25	15
11/24/42	0.287	2.781	-	2.2	-	65	30	0	0

continued on next page

TABLE VIII. - continued

Date	Ascorbic Acid per 100 ml. Blood mg.	Ascorbic Acid per 100 ml. Semen mg.	Sperm Conc. m/cc	Vol. c.c.	Semen pH	Per Cent Motility Fresh	Per Cent Motility 24 hr.	Per Cent Motility 48 hr.	Per Cent Motility 72 hr.
12/8/42	0.287	3.207	-	2.6	-	35	30	12	0
12/15/42	0.366	3.570	-	3.2	7.12	65	45	0	0
12/22/42	-	4.55	-	6.7	-	45	5	0	0
12/29/42	0.327	8.415	1.584	4.0	-	40	20	5	0
1/5/43	-	4.695	-	2.6	-	55	45	15	0
1/14/43	0.275	3.123	-	3.4	-	65	40	0	0
2/24/43	0.399	2.804	-	3.8	-	70	40	15	10
3/11/43	0.323	4.418	-	-	-	45	40	25	15
3/18/43	0.259	2.501	-	5.4	-	60	35	15	5
4/27/43	0.544	4.552	-	4.8	-	20	10	0	0

TABLE IX. PER CENT OF ABNORMAL SPERMATOZOA IN SEMEN OF "PATTY" BULL DURING SECOND TRIAL

Date	Per Cent Abnormal Sperm	Comments
4/12/42	76	Majority coiled tails
5/19/42	62	" " "
6/26/42	42	" " "
7/26/42	45	" " "
8/24/42	21	" " "
11/10/42	42	" " "

TABLE X. EFFECT OF CHLOROBUTANOL FEEDING TO BULL "1645" (HOLSTEIN).

Date	Ascorbic Acid per 100 ml.		Sperm Motility (Fresh)	Chlorobutanol treatment
	Blood mg.	Semen mg.		
1/17/41	0.28	0.88	None	-
1/22/41	-	-		Started feeding 5 grm.per day
1/27/41	0.30	0.288	+	Continuing
2/5/41	0.43	10.22	+	"
2/8/41	0.43	8.98	+ +	"
2/10/41				Discontinued
2/15/41	0.52	5.52	+ + +	
2/22/41	0.31	6.72	+ +	
3/1/41	0.36	8.30	+	
3/17/41				Started feeding 5 grm.per day
3/22/41	0.389	6.22	+ + +	Continued
3/29/41	0.495	4.08	+ + +	"
4/5/41	0.494	9.630	+ + +	"
4/12/41	0.435	7.77	+ + + +	Discontinued feeding
4/24/41	0.157*	9.21	+ + + +	
5/3/41	0.218	8.55	+ + + +	
5/17/41	0.342	10.63	+ + + +	
6/7/41	0.150	8.80	+ + + +	
9/24/41	-	5.36	+ + + +	
10/1/41	Start of second period - Started feeding 10 grams per day			
10/10/41	0.290	4.076	+ + +	Continued
10/17/41	0.250	2.502	+	"
10/24/41	0.475	5.316	+ +	"
11/7/41				Discontinued - lost muscle tone
11/8/41	0.191*	5.563	+ + +	
11/12/41				Started 5 grms. per day
12/2/41	0.356	7.407	+ + + +**	Continued
12/10/41	0.310	4.18	+ + + +	"
12/17/41	0.366	4.532	+ + + +	"
1/14/42	0.152*	8.495	+ + + +	"
1/23/42	0.151*	6.158	+ + + +	"
2/5/42	0.358	9.003	+ + + +	"
2/20/42	0.257	0.931	Bloody	"
3/14/42	0.290		+	Discontinued
3/17/42	0.290	1.256	+ + + +	Sample by massage
4/7/42	0.177	4.59	+ + + +	

* Low values due to hemolysis.
** Semen had 90 per cent motility when fresh and 50 per cent at 96 hours.

TABLE XI. EFFECT OF CHLOROBUTANOL ON BREEDING EFFICIENCY OF BULL "1645".

	Period	Treatment	Services	Conceptions	Services per Conception
1st.	11 mo.	None	42	11	3.80
1st.	5 mo.	275 gr.	13	5	2.60
2nd.	3 mo.	None	11	1	11.00
2nd.	4 mo.	1170 gr.	7	3	2.33

TABLE XII. EFFECT OF FEEDING CHLOROBUTANOL TO SIRE 72-A (HOLSTEIN).

Date	Ascorbic Acid per 100 ml.		Sperm Motility (Fresh)	Chlorobutanol Treatment
	Blood mg.	Semen mg.		
9/24/41	0.323	4.081	+ +	
10/1/41				Started feeding 10 gm. daily
10/10/41	0.125*	0.646 (1)	+ +	Continued
10/17/41	0.214	2.197	+ +	"
10/24/41	0.504	- (2)		"
11/8/41	0.224	4.186	+ + +	"
11/9/41				Reduced dosage to 5 gr. daily
12/2/41	0.276	1.935	+	5 gr. per day
12/10/41	0.308	-		" " " "
12/17/41	0.232	1.996	+ + +	" " " "
1/14/42	0.197	1.839	+	" " " "
1/23/42	0.273	- (3)		Fed 10 gr. for 9 days then 5 gr.
2/4/42	0.484	3.75	+ + + +	5 gr.
2/20/42	0.540	4.615	+ + + +	" "
3/14/42				Discontinued feeding
3/16/42	0.225	2.082	+	None
4/7/42	0.017	1.240	+ +	"
6/9/42	0.147	0.920	Few	"

* Slight hemolysis.
(1) Semen sample bloody.
(2) Not able to get sample due to lack of coordination.
(3) Semen thick, bloody mass - only 0.7 cc. in sample.

TABLE XIII. EFFECT OF CHLOROBUTANOL ON BREEDING EFFICIENCY OF SIRE 72-A.

Period	Treatment	Services	Conceptions	Services per Conception
3 mo. Prior	None	17	3	5.66
4 mo. of treat-ment	5-10 gr. per day	13	5	2.60

TABLE XIV. EFFECT OF CHLOROBUTANOL FEEDING TO SIRE 1601 (HOLSTEIN).

Date	Ascorbic Acid per 100 ml.		Sperm Motility (Fresh)	Chlorobutanol Treatment
	Blood mg.	Semen mg.		
3/1/41	0.22	8.55	+ + +	
3/7/41				Started feeding 5 gr. daily
3/22/41	0.27	8.44	+ + +	
3/29/41	0.117	7.21	+ +	
4/2/41				Discontinued feeding
4/5/41	0.119	8.806	+ +	
4/7/41	0.229			Started feeding 5 gr. daily
4/9-12/41				10 gr. daily for 3 days
4/12/41	0.313	10.60	+ + + +	Discontinued feeding

TABLE XV. EFFECT OF CHLOROBUTANOL ON THE BREEDING EFFICIENCY OF SIRE 1601.

Period	Treatment	Services	Conception	Services per Conception
1 mo.	None	6	2	3.00
4 mo.	1 mo. 5 gr. per day	15	11	1.36

TABLE XVI. EFFECT OF CHLOROBUTANOL FEEDING TO SIRE "SHORTHORN".

Date	Ascorbic Acid per 100 ml.		Sperm	Semen	Per Cent Motility - Sperms			
	Blood mg.	Semen mg.	Conc. m/cc	pH	Fresh	24 hr.	48 hr.	72 hr.
3/17/42	0.241	3.30	-	-	-	-	-	-
3/20/42	Started feeding 10 grams of chlorobutanol per day							
3/26/42	0.087	4.760			10	0	-	-
4/3/42	0.316	6.084			50	20	-	-
4/10/42	Fed last 10 gr. dose - none fed 4/8 and 4/9 - Incoordination							
4/17/42	0.244	1.994			50	10	-	-
4/23/42	0.168	3.908	1.312		70	20	-	-
4/28/42	0.222	3.315	.620	6.90	70	60	50	30
5/4/42	0.297	4.629	.140	7.50	40	0	-	-
5/7/42	0.313	4.260	.648	6.86	60	40	40	15
5/7/42	Started feeding 40 grams of chlorobutanol per week							
5/12/42	0.323	5.292	.860	6.78	75	10	10	-
5/19/42	0.384	2.815	.872	6.88	80	50	20	10
5/26/42	0.384	11.68	.792	6.78	90	30	-	-
5/26/42	Discontinued feeding chlorobutanol							
6/2/42	0.097	4.132	.788	6.60	80	40	30	15
6/9/42	0.227	4.060	.336	6.96	80	60	40	20
7/30/42	0.174	4.999	-	-	85	75	65	30

TABLE XVII. PER CENT OF ABNORMAL SPERMATOZOA IN THE SEMEN OF SIRE "SHORTHORN".

Date	Per Cent Abnormal	Comments
4/21/42	13	Bent bodies
5/19/42	11	Bent bodies and coiled tails
6/9/42	9	" " " " "
7/30/42	12	" " " " "

TABLE XVIII. EFFECT OF FEEDING CHLOROBUTANOL TO THE "WELLS" BULL (JERSEY).

Date	Ascorbic Acid per 100 ml.		Sperm Conc.	Semen		Per Cent Motility of Sperm			
	Blood mg.	Semen mg.	C/mm	pH	Vol.	Fresh	24 hr.	48 hr.	72 hr.
4/17/42	0.100	0.378	-	-	3.0	20	-	-	-
4/21/42	0.164	2.146		7.20	2.0	30	5	-	-
4/23/42	0.168	2.304	416	-	2.7	10	-	-	-
4/28/42	0.222	0.098	1380	7.48	2.5	50	20	10	5
5/4/42	0.349	0.732	608	7.32	3.0	60	5	-	-
5/7/42	0.190	4.600	888	6.98	1.7	50	30	10	0
5/7/42	Started feeding 40 grams of chlorobutanol per week								
5/12/42	0.340	1.186	1120	7.70	2.5	40	20	0	0
5/19/42	0.169	1.247	2240	7.50	2.2	30	0	0	0
5/26/42	0.264	0.837	837	6.42	2.8	20	10	0	0
5/28/42	Increased to 20 grams every other day								
6/2/42	0.106	1.058	504	6.86	2.0	30	20	5	0
6/9/42	0.182	1.383	368	7.54	3.0	40	20	0	0
6/9/42	Increased from 20 grams to 30 grams every other day								
6/17/42	Showed incoordination - discontinued chlorobutanol								
6/26/42	Started feeding 10 grams every other day								
7/1/42	0.345	0.738		7.78	3.5	10	0	0	0
7/7/42	0.641	1.782		6.92	5.0	90	40	not read	
7/15/42	0.397	2.883		7.50	2.5	65	30	not read	
7/22/42	0.394	5.640		7.18	2.4	80	75	15	0
7/28/42	0.520	4.941		-	0.7	85	50	sample lost	
8/4/42	0.496	3.227		7.3	2.5	70	60	-	0
8/10/42	0.467	3.823		-	3.0	60	0		
8/17/42	0.345	4.743		7.2	3.2	25	0	0	0
8/17/42	Discontinued chlorobutanol								
8/24/42	0.314	3.570		6.82	2.0	65	55	lost sample	
8/31/42	0.352	3.090		7.38	2.0	25	25	1	0
9/4/42	0.200	2.715		7.00	3.5	80	55	25	5
9/12/42	0.314	2.607		-	3.3	80	55	40	20
9/21/42	0.288	3.493		-	1.4	70	55	5	0
9/29/42	0.370	3.188		-	2.0	15	10	1	0
10/15/42	cloudy	2.772		-	1.4	20	15	0	0
10/20/42	0.427	3.991		-	2.6	65	30	20	5
10/27/42	0.327	3.270		6.95	3.7	80	30	15	2
11/3/42	-	2.356		7.09	2.4	85	55	5	2
11/10/42	Sold								

TABLE XIX. EFFECT OF CHLOROBUTANOL ON BREEDING EFFICIENCY OF "WELLS" SIRE.

Period	Treatment	Services	Conceptions	Services per Conception
3 months	None	10	2	5.00
2 months	390 grams	3	2	1.50

TABLE XX. PER CENT OF ABNORMAL SPERMATOZOA IN SEMEN OF WELLS BULL.

Date	Per Cent Abnormal	Comments
4/21/42	14	Coiled tails
5/5/42	14	" "
6/9/42	16	" "
7/15/42	19	" "
8/24/42	12	" "

TABLE XXI. EFFECT OF CHLOROBUTANOL FEEDING ON SIRE "D-520" (RED DANE).

Date	Ascorbic Acid per 100 ml. Blood mg.	Ascorbic Acid per 100 ml. Semen mg.	Sperm Conc. C/mm	Semen pH	Semen Vol.	Per Cent Motility of Sperm Fresh	24 hr.	48 hr.	72 hr.
6/15/42	0.139	-	-	-	-	-	-	-	-
6/20/42	0.334	filtrate destroyed				No motile sperms; considerable sediment			
6/20/42	Started feeding 20 grams chlorobutanol by capsule								
6/29/42	0.324	-				Appeared sleepy			
6/29/42 to 7/6/42 fed 150 grams of chlorobutanol by 30 gram doses in capsule form over a 5 day period. Drowsy during this period									
7/7/42	0.328	0.245		6.42	1.3	No sperms			
7/15/42	0.257	1.127		7.60	2.0	No sperms			
7/15/42	Started feeding 10 grams chlorobutanol every other day								
7/22/42	0.448	1.998		6.92	13.3	10	5	0	0
7/28/42	0.385	1.532		7.10	2.9	15	5	0	0
8/4/42	0.332	2.012		7.22	7.0	Few dead sperm			
8/10/42	0.371	3.000		-	3.6	" " "			
8/17/42	0.392	2.224		7.00	3.3	" " " . Semen brown in color			
8/17/42	Discontinued feeding chlorobutanol								
8/24/42	0.402	2.130		6.78	2.5	No sperms			
8/31/42	0.255	3.444		8.96	1.5	Few dead sperms			
9/4/42	0.137	-		-	2.3	2	2	2	0
9/12/42	0.261	3.140		-	-	Dead sperm			
9/15/42	0.226	Fed 25 cc. of shark liver oil containing 25,000 I.U. of vitamin A.							
9/19/42	Increased to 50 cc. shark liver oil every 3 days								
9/21/42	0.332	2.792	No motility. Considerable cell debris.						
9/29/42	0.267	Masturbated							
10/2/46	-	"							
10/15/42	0.295	2.682			1.8	Few dead sperms			
10/10/42	Started 25 grams chlorobutanol twice a week								
10/20/42	0.362	4.450			2.2	Many dead sperms			
10/27/42	0.363	3.994		7.08	0.7	" " "			
11/10/42	0.242	3.583		7.12	3.3	Cell debris and crystals			
11/17/42	0.323	3.495			0.6	" " " "			
12/1/42	0.254	2.709			0.4	" " " "			
12/8/42	0.279	2.245		6.95	9.6	Seminal fluid			
12/12/42	Started feeding 50 grams chlorobutanol 2 times per week								
12/17/42	Was too drowsy to collect sample								
12/22/42	0.334	Was too drowsy to collect sample							
1/5/43	0.230	0.870	Seminal fluid						
1/5/43 to 1/12/43 - No chlorobutanol									
1/13/43	-	3.583	Few club tailed sperm						
1/13/43	Started feeding 5 grams chlorobutanol per day								
1/20/43	-	-			1.9	Few dead sperm			
1/27/43	-	3.210				" " "			
1/27/43	Stopped feeding shark liver oil								
2/6/43	Stopped feeding chlorobutanol								
2/10/43	0.318	2.990			0.4	No sperm			
2/24/43	0.364	3.275			6.4	" "			
3/11/43	0.286	2.756			1.7	Few dead sperms			
3/18/43	0.231	1.031				" " "			
4/6/43	-	4.204				Seminal fluid - no sperms			

TABLE XXII. EFFECT OF CHLOROBUTANOL FEEDING ON SIRE "THORNE" (JERSEY).

Date	Ascorbic Acid per 100 ml.		Semen		Per Cent Motility of Sperm			
	Blood mg.	Semen mg.	pH	Vol.	Fresh	24 hr.	48 hr.	72 hr.
10/10/42	-	2.977	-	4.5	90	60	45	20
10/22/42	0.225	4.422	-	3.4	90	65	45	30
10/29/42	-	2.632	Started feeding 10 grams chlorobutanol per day					
11/3/42	-	2.343		3.6	60	45	40	30
11/10/42	0.286	lost		3.2	50	35	30	20
11/12/42	-	3.655		4.1	85	50	40	35
11/21/42	Muscular incoordination. No chlorobutanol from 11/21 to 11/28							
12/1/42	0.219	2.709	7.95	3.2	30	15	0	0
12/15/42	0.366	2.520	7.20	2.8	30	15	0	0
12/15/42	-	2.871	7.05	4.2	55	45	40	30
12/22/42	-	5.041	-	4.1	75	5	0	0
1/5/43	0.341	No semen sample taken						
1/5/43	to 1/12/43 Muscular incoordination. No chlorobutanol							
1/6/43		4.390	-	4.2	80	35	25	0
1/11/43		6.745	-	3.8	85	45	40	30

TABLE XXIII. EFFECT OF CHLOROBUTANOL ON BREEDING EFFICIENCY OF "THORNE" BULL.

Period	Treatment	Services	Conceptions	Services per Conception
3 mo. previous	None	13	3	4.33
3 mo. following	61 days	7	5	1.40

TABLE XXIV. EFFECT OF CHLOROBUTANOL FEEDING ON SIRE "LADD" (JERSEY)

Date	Ascorbic Acid per 100 ml.		Sperm	Per Cent Motility of Sperm			
	Blood mg.	Semen mg.	Vol. ml.	Fresh	24 hr.	48 hr.	72 hr.
1/28/44	no	no	5.5*	20	0	0	0
1/28/44	Determination.	Started feeding 5 grams chlorobutanol per day					
2/16/44	made		3.0	45	20	0	0
2/17/44			3.5	50	20	0	0
2/25/44			4.5	50	40	25	20
3/3/44			3.5	60	45	10	0
3/16/44			4.6*	75	50	40	15
4/19/44			4.0*	10	0	0	0
4/22/44			3.4	40	0	0	0
4/22/44	Discontinued feeding chlorobutanol						

*Two ejaculates each time.

TABLE XXV. EFFECT ON THE BREEDING EFFICIENCY OF THE "LADD" SIRE.

Period	Treatment	Services	Conception	Services per Conception
3 mo. previous	None	9	1	9.00
3 mo. following	425 gm. in 85 days	15	2	7.50

TABLE XXVI. EFFECT OF CHLOROBUTANOL FEEDING ON REPRODUCTION IN DAIRY COWS. HERD "A".

Cow	Weight Lbs.	Sires Used No.	Services Before Treatment No.	Dosage per Day Grams	Days Fed	Services per Conception No.
Surprise	850	3	6	5	21	1
Joy	600	3	9	5	21	(5)
Joy				20	5	(1)
Joy				5	16	6
Maid	800	3	6	5	21	1
Jewel	850	4	14	5	21	(6)
Jewel				20	5	(1)
Jewel				5	16	7
Bramble	850	3	5	5	21	1
Marcia	900	3	8	5	21	(3)
Marcia				20	5	(1)
Marcia				5	16	4
Vera	900	1	5	5	21	1
Naidna	850	2	5	5	21	1
Valiant Girl	800	3	8	5	21	(5)
Valiant Girl				20	5	(2)
Valiant Girl				5	16	7

TABLE XXVII. EFFECT OF NORMAL AND SHOCK TREATMENT ON MILK PRODUCTION, HERD "A".

Cow	Type of Treatment	Grams in Dosage	Lbs. of milk Before Treatment (per day)	Lbs. of Milk During Treatment (per day)
Surprise	Normal	5	35	35
Maid	"	5	25	20
Bramble	"	5	35	32
Vera	"	5	37	35
Naida	"	5	35	37
Average			33.5	31.8
Jubilee	Shock	20	21	10
Valiant Girl	"	20	20	8
Average			20.5	9.0

TABLE XXVIII. EFFECT OF CHLOROBUTANOL ON REPRODUCTION IN DAIRY COWS, HERD "B".

Cow No.	Weight	Date of Service	Service	Sire	Remarks
652	1285	9/11/44	1	600-G	
Brown		11/9/44	2	600-F	
Swiss		12/20/44	3	600-G	
		3/4/45	4	600-F	
		3/25/45	5	600-E	
		4/17/45	6	600-E	
		4/28/45	7	600-E	
		5/11-6/19			Fed 5 grams per day
		5/31/45	8	600-E	Pregnant
640	1175	3/29/45	1		
Brown		4/18/45	2	600-E	
Swiss		5/9/45	3	600-E	
		5/30/45	4	600-E	
		5/30-6/19			Fed 5 grams per day
		6/14/45	5	600-E	Pregnant
621	1377	3/7/45	1	600-E	
Brown		3/29/45	2	600-E	
Swiss		4/17/45	3		
		5/11-6/19			Fed 5 grams per day
		5/31/45	4	600-E	Pregnant
J-4	735	2/2/45	1	300-Q	Tried ascorbic acid
Jersey		2/7/45	2	300-Q	
		2/27/45	3	300-Q	
		4/11/45	4	300-Q	
		5/12-6/19			Fed 5 grams per day
		5/30/45	5	300-Q	Pregnant

TABLE XXVIII - Continued

Cow. No.	Weight	Date of Service	Service	Sire	Remarks
J-5	766	1/22/45	1	300-Q	
Jersey		2/10/45	2	300-Q	
		2/14/45	3	300-Q	
		3/5/45	4	300-Q	
		3/25/45	5	300-Q	
		5/12-6/19			Fed 5 grams per day
		6/8/45	6	300-Q	Pregnant
393-X	1130	4/12/45	1	300-Q	
		5/2/45	2	300-Q	
		5/23/45	3	300-Q	
		6/21/45	4	300-Q	
		5/26-6/19			Fed 5 grams per day
		8/1/45	5	300-Q	Pregnant
J-2	908	1/31/45	1	300-Q	Tried ascorbic acid
Jersey		2/19/45	2	300-Q	
		3/26/45	3	300-Q	
		4/19/45	4	300-Q	
		5/9/45	5	300-Q	
		5/12-6/19			Fed 5 grams per day
		5/30/45	6	300-Q	Pregnant
J-3	800	2/1/45	1	300-Q	Tried ascorbic acid
Jersey		2/22/45	2	300-Q	
		3/15/45	3	300-Q	
		4/19/45	4	300-Q	
		5/12-6/19			Fed 5 grams per day
		6/15/45	5	300-Q	Pregnant
A-1	1285	3/7/45	1	400-K	
Ayrshire		4/17/45	2	400-K	
		5/11-6/19			Fed 5 grams per day
		5/30/45	3	400-K	
		6/9/45	4	400-K	Pregnant
634	1308	3/28/45	1	600-E	
Brown Swiss		4/17/45	2	600-E	
		5/9/45	3	600-E	
		5/11-6/19			Fed 5 grams per day
		5/30/45	4	600-E	
		6/26/45	5	600-E	Pregnant
650	1250	12/16/44	1	Judd's Bridge	
Brown Swiss		1/6/45	2	Demonstrator	
		1/27/45	3	Demonstrator	
		3/7/45	4	"	
		3/29/45	5	600-E	
		4/18/45	6	600-E	
		5/11-6/19			Fed 5 grams per day
		5/23/45	7	600-E	
		6/22/45	8	600-E	Pregnant

TABLE XXIX. EFFECT OF CHLOROBUTANOL ON REPRODUCTION IN DAIRY COWS, HERD "C".

Cow No.	Weight	Date of Service	Service	Sire	Remarks
A-27	955	1/24/44	1	Prilly	
Holstein	982	2/15/44	2	"	
	972	3/6/44	3	"	Started 3 gm. chlorobutanol daily
	954	3/24/44	4	"	Pregnant 3/8/44
A-29	1165	6/3/44	1	Fairfield	
Holstein	1050	7/16/44	2	Paul	
	1099	8/5/44	3	"	
	1064	8/25/44	4	"	Started 4 gm. chlorobutanol 9/30/44
	1038	9/15/44	5	"	
	1123	10/28/44	5	Fairfield	Pregnant
A-31	942	2/15/44	1	Prilly	
Holstein	954	3/6/44	2	"	Started 3 gm. chlorobutanol per day
	978	3/24/44	3	Fairfield	3/8/44
	980	4/13/44	4	"	Pregnant-Aborted 11/15/44
A-37	1020	2/29/44	1	Fairfield	
Holstein	1063	4/4/44	2	"	Started 3 gm. chlorobutanol daily
	1050	4/26/44	3	Paul	5/10/44
	1100	6/8/44	4	"	Pregnant
A-39	896	12/31/43	1	Prilly	
Holstein	985	3/14/44	2	Fairfield	
	1017	4/14/44	3	"	
	1010	5/4/44	4	Paul	Started 3 gm. chlorobutanol daily
	1025	5/20/44	5	Fairfield	Pregnant 5/10/44
66	725	1/11/44	1	Thorne	
Jersey	705	4/10/44	2	Ladd	
	700	5/3/44	3	Thorne	
	760	5/27/44	4	"	Started 3 gm. chlorobutanol daily
	750	7/31/44	5	Design	Pregnant 5/10/44
412	1055	3/24/44	1	Fairfield	
Holstein	1057	4/14/44	2	"	
	1050	6/13/44	3	Paul	Started 3 gm. chlorobutanol daily
	1045	7/14/44	4	Fairfield	Pregnant 6/15/44
288	1509	9/21/44	1	Fairfield	
Holstein	1511	10/16/44	2	Paul	
	1557	11/4/44	3	"	Fed 4 gm. chlorobutanol daily
	1605	1/12/45	4	"	11/8/44 to 1/25/45
	1606	2/24/45	5	"	
	1597	3/19/45	6	"	Started 8 gm. chlorobutanol daily
	1596	4/12/45	7	"	Pregnant 3/30/45

TABLE XXX. EFFECT OF CHLOROBUTANOL ON REPRODUCTION IN DAIRY COWS, HERD "D".

Cow	Weight	Number of Sires Used	Services before Treatment	Doses per Day Grams	Days Fed	Number of Services to Conceive
Sadie I	1350	2	4	5	35	1
Snowball	1000	1	4	5	24	1
Admiral's Clara	1100	2	1	5	20	(3)
" "			5		31	(2)
" "				5	24	6
Admiral's Mary	1000	2	4	5	45	2

( ) Number of times bred without conceiving after feeding for days shown

TABLE XXXI. EFFECT OF CHLOROBUTANOL FEEDING ON PLASMA ASCORBIC ACID AND LIBIDO

Sire	Date	Plasma Ascorbic Acid Mg. per cent	Service Time	Chlorobutanol Dosage
Patty	2/28/41	.22	10-15 min.	None
	3/7/41	.28	5 "	5 gm. started 2/28/41
	3/14/41	.41	1 "	" "
	3/22/41	.51	5 sec.	"
	3/29/41	.27	5 "	"
Pheiffer Boy	12/12/41		1 hr.	None
	12/13/41	.338		10 gm. started 12/12/41
	12/30/41	.530	15 min.	" "
	1/3/42		5 "	None