CYTOGENETIC AND HERD ANALYSIS STUDIES IN BEEF CATTLE

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ABSTRACT

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IN BEEF CATTLE

By

Harold Dee Woody

Modification of previously established techniques of whole blood leukocyte culture has resulted in satisfactory preparations for chromosome studies. By culturing whole blood leukocytes in a formulated culture medium in the presence of phytohemagglutinin, optimum leukocyte growth has been present. Hypotonic treatment in 0.9% sodium citrate, prolonged fixations of methanol-glacial acetic acid (3:1), followed by a fixative solution of 45% glacial acetic acid and slide preparation by air drying, has provided a maximum number of well spread metaphases. These modifications have aided in the establishment of a reliable and consistent technique for cytogenetic studies in cattle.

With this technique modification, a cytogenetic study was completed to examine the presence of chromosomal abnormalities in cattle. By peripheral blood leukocyte culture and metaphase slide preparation, the chromosomal complement of 100 cows was observed. The chromosome complement of each cow was evaluated by visual observation and karyotype.

Results of the 100 cows examined showed one cow expressed a significant abnormal chromosome complement of 58. This cow had previously given birth to a normal bull calf. The calf showed no chromosomal abnormality when examined.

Three cows with histories of offspring expressing phenotypic disorders showed no abnormality in their chromosome complement when examined.

The reproductive performance of 200 purebred Angus cows and 100 crossbred cows was analyzed from individual breeding records. Individual records of each cow were recorded for computer analysis and visual observation.

The average reproductive performance parameters observed in the 200 purebred Angus cows were: number of services per conception, 2.79; interval from calving to first breeding, 80.9 days; interval from first breeding to conception, 67.2 days; and calving interval, 424.6 days. There was no relationship found between age of dam, services per conception and calving interval. The younger dams showed a longer interval from calving to first estrus than the older dams.

In analyzing the breeding records in a herd of 100 crossbred cows the average reproductive performance was summarized. The results were: average services per conception, 1.35; interval from calving to first breeding, 70.5 days; interval from first breeding to conception,

9.6 days; and calving interval of 371.4 days. Older dams showed a higher conception rate but had a longer interval from calving to first breeding than younger dams. Lower services per conception were shown for dams receiving higher energy levels.

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PART I

CYTOGENETIC STUDY IN BEEF CATTLE

INTRODUCTION

The presence of chromosomal anomalies has been prevalent in cattle for years. Numerous cytogenetic studies have reviewed various disorders in cattle. These studies of phenotypic variations have revealed abnormal chromosome complements present. The chromosomal abnormalities have a bearing on fertility levels and production in cattle. Further studies are needed in analyzing the chromosome complement in cattle in relationship to reproductive efficiency.

Present procedures for cytogenetic studies have been derived from methods used in studying human disorders. These procedures are not completely dependable for securing optimum results for chromosome analysis in cattle. Research is needed for establishment of a reliable and consistent technique for cytogenetic studies.

Objectives of these investigations included:

1. Development of a reliable and consistent technique for cytogenetic studies in cattle.
2. Analyzing the chromosome complement in a herd of cattle.
3. To observe the relationship of chromosome abnormalities between the dam and offspring.

LITERATURE REVIEW

Chromosome Analysis

Chromosome analysis in domestic cattle (Bos taurus) dates back to works of Bardeben in 1892, but Krallinger in 1927 was the first to report the exact number of chromosomes in cattle, as reviewed by Makino (1957). Lillie (1916) used cytogenetics extensively in studying the freemartin syndrome in cattle. These earlier reports were further supported by Makino (1951) in examining 3,317 species in the Atlas of the Chromosome Numbers in Animals.

In a more recent study, Wurster and Bernirschke (1968) reported the chromosome morphology in 50 species. Evidence reported by Ohno (1969) suggests that the X chromosome has remained essentially the same since establishment in early vertebrate evolution, possibly more than 300 million years ago.

Painter (1921) studied sex determination and noted common variations of XX (female) and XY (male) between individuals. Following Painter's discovery, Barr and Bertram (1949) found a chromatin mass (Barr body) in cells with XX present. In this study it was noted that animals containing XX had one chromatin mass while the XY had no chromatin mass present.

On the basis of morphology, sex differentiation is dependent upon a single pair of chromosomes, the X and Y (Melander, 1959; Ulbrich, 1963). The chromosome complement in cattle consists of 29 pairs of autosomes (acrocentric) and one pair of sex chromosomes (metacentric). Studies by Kieffer and Cartwright (1967), and Bhambhani and Kuspira (1969), have shown cytogenetic similarities between Bos taurus (U.S. domestic cattle) and Bos indicus (brahman or zebu). In B. taurus, the X chromosome is the largest of the karyotype with equal length of the arms. The Y chromosome is 3-5 times smaller than the X and is the smallest of the karyotype. There is a morphological difference between the Y chromosome in the B. indicus and the B. taurus. In the B. indicus the structural arm is shorter and is not as easily distinguished from autosomes. The X chromosomes of the females in both B. taurus and B. indicus are morphologically similar.

Similar studies were performed to compare the American bison (B. bison) and domestic cattle by Basrur and Moon (1967) and Bhambhani and Kuspira (1969). On the basis of chromosomal structure between the karyotypes of the two species, similarity is restricted to the autosomes (acrocentric) and X chromosomes (metacentric). The X chromosome, as previously discussed, is metacentric in the B. taurus. In contrast, the Y chromosome is telocentric with

a short arm in both the B. bison and the B. indicus. Similarities in numbers and morphology suggest that the B. taurus, B. indicus, and B. bison are closely related, possibly evolving from a common ancestor.

One of the first major advancements in cytogenetics occurred when Murray and Kopech (1953) developed an in vitro cell culture. These cultured cells, when placed into a hypotonic solution, tend to swell and the chromosomes become clearly visible after staining (Hsu, 1952).

The recent development of refined leukocyte culture allows investigators to view various chromosome anomalies in domestic animals. Moorhead et al. (1960) discovered a reliable and consistent method of leukocyte culture in humans that may be easily altered for domestic animals. This technique involves adding phytohemagglutinin and induction of leukocyte growth which is supported in tissue culture medium 199 (TC-199) and homologous plasma. Colchicine, in a 6-hour treatment, is added after 66 hours of culture to prevent spindle formation and arrest cell division at metaphase. Cells are suspended in Earle's balanced salts and centrifuged for leukocyte separation. Cell permeability is increased by the hypotonicity of distilled water and a fixative solution of 1:3 of glacial acetic acid to methyl alcohol.

In a modification of Moorhead's technique for cytogenetic studies in cattle, observations have been made

by Nichols et al. (1962), Herschler et al. (1962), Pfeiffer (1963), Basrur and Gilman (1964), and Dunn (1970). These authors found adequate results in a technique similar to Moorheads et al. (1960). Bhambhani and Kuspira (1969), when using 0.8% sodium citrate in contrast to distilled water, found an increased number of well spread metaphases in slides observed. Prolonged hypotonic treatments of distilled water caused breakage and damage to the cells in solution.

Basrur and Gilman (1964), later supported by Dunn in 1973 (personal communication), found that by adding 0.7 ml. of 7.5% sodium bicarbonate/100 ml. culture medium, contamination was reduced to a minimum during the incubation process. Sodium bicarbonate aids in maintaining the proper pH level during this period. Basrur and Gilman (1964) also used Carnoy's solution for cell suspension prior to slide preparation. They reported that a temperature of 37°C and 5% CO_2/air were optimum for culture conditions and leukocyte growth.

Because of failure of adequate leukocyte growth with phytohemagglutinin and blood culture, investigators have resorted to tissue culture (Hsu and Kellogg, 1960) or bone marrow (Bernirschke and Brownhill, 1962). Prolonged centrifugation has also aided in leukocyte separation (Sasaki and Makino, 1962; Ulbrich et al., 1963).

One of the first pioneers in slide presentation of chromosomes was Hsu (1952). After hypotonic treatment and fixation, cells were placed on slides, dried and stained in Giemsa solution. Rothfels and Siminovitch (1958) and Ford and Woollam (1963), found favorable results with the air drying method of preparation. In a comparison of air and blaze drying technique, Scherz (1962) found a large number of well spread metaphases when using the blaze drying method.

Using various technique modifications, numerous cytogenetic studies in cattle have investigated the freemartin syndrome. Lillie (1916), Chapin (1917), and Willier (1921), were the first to realize that in twins born of opposite sex, the female's reproduction system may be suppressed and male characteristics prevail. Owen (1945) studied the mechanism of the freemartin syndrome and found identical blood types between both twins, these differing from either parent. More recent studies by Fechheimer et al. (1963), Muramoto et al. (1965), Makino et al. (1965), Kanagawa et al. (1965), Herschler et al. (1966), and Short et al. (1969) have presented evidence by chromosome analysis that freemartinism is associated with anastomosis of the chorionic blood vessels of the twins in utero.

After observing numerous animals Kanagawa et al. (1965) found cases of cell chimerism in freemartins and their co-twins. Goodfellow (1965) in chromosome studies

in two pairs of bovine twins discovered 70% XY (male) cells present in one freemartin studied and 56% (XY) in the second heifer. Basrur and Kanagawa (1969) performed chromosome studies on 22 pairs of heterosexual twins and found that all showed normal XY or XX cells present, except for two pairs observed. In one pair, the bull expressed an equal number of male and female cells, while in the second pair, the bull twin exhibited a higher percent of female cells. Basrur (1966) also examined quintuplets by peripheral blood and chromosome analysis for evidence of cell chimerism. In three of the quintuplet males studied, XX cells present averaged 27% while 60% XY cells were present in the females.

Cell chimerism in the freemartin and bull twin is further supported by Ohno (1962), Teplitz et al. (1967), Dunn et al. (1968) and Short et al. (1969). The freemartin syndrome occurs in 90% of cases where heifers were born co-twin to a bull, as noted by previous authors.

The fertility level of the male twin was measured by Stafford (1972) in three bulls born twin to freemartins. Two of these expressed no abnormal values while the third expressed poor semen quality and a low 30-60 day nonreturn rate. Makino (1962), in conflict with other authors, detected no chromosomal abnormalities in either freemartins or bull twins. In summation of the data analyzed, it appears

that examination of cultured leukocytes for sex chromosome chimerism is a reliable test for diagnosing the freemartin syndrone in cattle.

In a cytogenetic study of 10 breeds of cattle, Harvey (1971) discovered an autosomal translocation and abnormal DNA replication in one bull observed. Chromosome studies were carried out on 69 bulls from the 10 breeds of cattle. One Charolais bull examined was found to be a translocation heterozygote. Eight of the twenty crossbred calves sired by this bull had a similar condition. In a similar study, Gustavsson et al. (1969) studied 1,145 Sweedish Red and White cattle and 14% were found to have cases of acrosomal translocations. One heifer in this study expressed a normal female appearance and regular estrous cycles. After nine artificial insemination services the heifer conceived, but the calf was born dead. Analyzing abnormal chromosome complements in cattle may be an aid in overcoming low fertility rates.

Reick (1970) discovered an X-trisomy (XXX) in a Fleckvieh heifer. The sexual function of the heifer was normal but in phenotypic appearance, she excelled in the lumbar column. Upon analysis of her pedigree and cytogenetic studies, other descendants expressed similar meiotic disturbances.

Inbred lines of cattle increase in homozygosity and usually experience a decline in production and reproductive

performance (Herschler et al., 1962; Zartman and Fechheimer, 1967). Zartman and Fechheimer (1967) studied abnormal chromosome complements due to inbreeding in Hereford cattle by leukocyte culture. The frequency of polyploid cells (< 60) present was 7.2%. Peridiploid cells were present in 16.6% of the cases with a range of 56 to 62 chromosomes present. In support of this Hauschka et al. (1962) reported nondisjunction to be inherited in certain human families. These results are indicative of the influence of inheritance upon abnormal mitotic occurrences.

Dwarfism in the bovine has been described by Johnson et al. (1950), Gregory et al. (1951, 1955), Koger et al. (1955) and Leuchtenberger (1956). The dwarfism syndrome is an autosomal recessive trait and is studied through known dwarfs or dwarf carriers. Leuchtenberger et al. (1956) and Benjamin and Stringham (1969) in cytogenetic studies of dwarfism, found no chromosomal abnormalities present. This information supports the theory that dwarfism is a genetic inheritance and not a chromosomal anomaly.

Chromosome analysis has also been used extensively in studies of intersexuality in the bovine by McFeely et al. (1966, 1967), Dunn et al. (1968, 1970), and Kieffer and Sorensen (1970, 1971). Intersexuality, as explained by Dunn et al. (1968), may be caused by: (1) intrauterine exchange of cells between heterosexual twins, (2) early

mitotic errors in an XY zygote, and (3) fertilization of an ovum and its polar body or two ova, with subsequent fusion. The third mechanism explains the XX/XY chimerism in human cases (Corey et al., 1967). Dunn et al. (1970) revealed XXY cells in a bovine hermaphrodite when examining 400 metaphases prepared through tissue culture. This triploid case is analogous to Klinefelters syndrome in humans (Jacob and Strong, 1959).

Recent studies of intersexuality have been studied in other species by chromosome analysis. In the pig, Vogt (1966, 1967), Breeuwsma (1968), Maik (1969) and Smith and Marlowe (1971) have studied various cases of intersexuality. Vogt (1966) observed 46 of 50 metaphases from an intersex pig and found a normal diploid chromosome number of 38, with the remaining four cells of 37 or 39 chromosomes present. Maik (1969) studied 25 cases of intersexes in the pig and found 17 cases of pseudo-hermaphroditism. In a similar study, White (1969) found a rare case of intersex in a horse with various male and female organs, but no cytogenetic studies were completed.

Bruere et al. (1969) discovered testicular hypoplasia and atrophy of testicular tissue in two sheep of different breeds. A cytogenetic study resulted in a chromosome number of 55 (XXY) in all tissues studied. Similar findings have been reported in 5% of rams observed (Gunn et al., 1942) and Swedish Highland bulls (Langerlof, 1942).

Chromosome abnormalities in early embryos of the pig have been cited by Hanley (1961), McFeely (1966, 1967) and Smith and Marlowe (1971). Hanley (1961) reported that 33% of the embryos die during the first half of gestation. McFeely (1966) recovered 8 blastocysts and revealed that 7 of these possessed a normal number of chromosomes. In the eighth blastocyst, all but two of the cells were triploid. In a later study, McFeely (1967) recovered 88 blastocysts from 7 gilts on the tenth day of gestation. Chromosome analysis of the blastocysts showed that 10% of the cells studied had chromosomal abnormalities. Smith and Marlowe (1971) investigated chromosome abnormalities of 25-day old embryos. Of 797 cells counted, 719 contained a normal diploid chromosome number of 38 while the remaining cells (9.8%) had abnormal chromosome numbers. These data suggest that chromosomal abnormalities account for a large percentage of the early embryonic mortality in pigs.

Complete studies have recently been performed with abnormal chromosome complements in rabbit blastocysts. In a recovery of 6-day blastocysts, Martin and Shaver (1972) found a structural anomaly of the Y chromosome in 58% of the blastocysts. Similar morphological abnormalities have been noted in man (Cohen et al., 1966), hamster (Lehman et al., 1963), rat (Hungerford and Nowell, 1963), and deer mice (Arakaki and Sparkes, 1963).

Recently in rabbits, Shaver and Carr (1967) and Shaver (1970) found that when fertilization was delayed by human chorionic gonadotropin, administered at varying intervals prior to mating, there was an increase in chromosomally abnormal blastocysts. In a similar study, Shaver and Carr (1969) reported an increase in triploidy (XXY) of the blastocysts examined. Chromosomal abnormalities in the early stages of development were found by Hansen-Melander and Melander (1971) when blastocysts were smaller than normal in size.

Investigators have related chromosomal anomalies to embryonic death, abortions and abnormalities in humans. Carr (1965) and Makino (1967) have reported cases in human abortions attributable to chromosomal disorders. Abnormal chromosome complements found in Klinefelters disease (XXY) have also been observed by Jacobs and Strong (1959) and Ferguson-Smith (1958). Further human disorders have been observed in Turners syndrome (XO), as cited by Ford et al. (1959).

MATERIALS AND METHODS

In this chromosomal study in beef cattle, 100 Angus-crossbred cows from the dry-lot project at the Michigan State University Beef Cattle Research Center, were tested. Blood samples (5-8 ml.) were drawn from the jugular vein into 10 ml. sterilized syringes (20 ga.-1.5 inch needle) containing 0.5 ml. of sodium heparin as an anticoagulant. Each sample was held at room temperature for 1-3 hours to allow formation of a buffy coat.

Culturing.--A culture medium was prepared by the method of H. O. Dunn (personal communication) in a 100 ml. formulation. The medium consisted of the following: 80 ml. tissue culture medium 199 (Grand Island Biological Co., #115EE), 20 ml. fetal calf serum (Grand Island Biological Co., #614), 0.7 ml. sodium bicarbonate, 0.1 ml. Combiotic (Jensen-Salsbery Laboratories), and 5 ml. of phytohemagglutinin (General Biochemicals). Using a Pasteur pipette, 10 ml. of culture media were added to each sterilized culture bottle. One ml. of the plasma from the syringe was expelled into two culture bottles containing culture media (0.5 ml./bottle). Duplication of the blood culture samples

was necessary to prevent losses due to contamination during the incubation period.

The culture bottles were capped over an open flame and mixed gently. Culture bottles were incubated for 72 hours at 37°C with 5% CO_2/air present.

Recovery of leukocytes.--Three hours prior to recovery, three drops of colchicine (50.0 µg/ml.--Sigma Chemical Co.) were added to each culture sample to inhibit spindle formation and stop mitotic division at metaphase. The cultures were mixed gently and incubated for a final three-hour period.

After three hours exposure to colchicine, the cultures were removed from the incubator, mixed gently and added to 12 ml. conical centrifuge tubes. The samples were centrifuged immediately at 735 rpm. (Clay-Adams centrifuge model CL, rotor no. 809) for five to eight minutes.

The supernatant was decanted from each centrifuge tube with a pipette. For hypotonic treatment, 5 ml. of 0.9% sodium citrate was added to each centrifuge tube and mixed thoroughly. After 10-15 minutes in the hypotonic solution the cells were recentrifuged at 735 rpm. for five to eight minutes.

The supernatant was again discarded from each centrifuge tube with a pipette. Freshly prepared fixative solution (4 ml.) consisting of methanol and glacial acetic

acid (3:1), was added slowly to each centrifuge tube and mixed by pipetting. After one hour of fixation, the samples were recentrifuged for five to eight minutes at 735 rpm. This procedure of fixation, decantation and centrifugation was repeated two more times.

After adding the third fixation of methanol--glacial acetic acid, the cells were resuspended. The centrifuge tubes were covered with parafilm to prevent evaporation and refrigerated for a period of 18 to 24 hours. Samples were removed and centrifuged immediately at 735 rpm. for five to eight minutes and the supernatant discarded.

A fixative solution (4 ml.) consisting of 45% glacial acetic acid and 55% distilled water, was added slowly to each centrifuge tube and mixed. After 10 to 15 minutes, the tubes were centrifuged and the supernatant discarded, as done previously.

Precleaned glass slides were labeled and heated on a hot-plate at 50°C. Following heating, the suspension of cells (0.5 ml.) was added to each glass slide and allowed to air dry. As an aid in optimum slide preparation, the hot-plate was slightly tilted causing dust collection at one end of the glass slide. Immediately following air-drying, the slides were dipped into methanol and stained for five minutes in a solution containing 6.0 ml. Giemsa stain (Allied Chemical), 0.3 ml. of 0.15 N ammonium hydroxide,

and 40 ml. of distilled water. The slides were then rinsed and allowed to dry.

The slides were examined with a Zeiss Aristophot II at a magnification of 800X, using cedar wood oil for clearer observation. On an average, 30 metaphases were observed and chromosome numbers counted for each cow examined. From these metaphases, three to five well spread metaphases were photographed at a magnification of 1000X. These photographs were enlarged threefold and a karyotype was prepared by arranging the chromosomes in pairs. In cattle, all 58 autosomes are acrocentric, while the sex chromosomes are metacentric.

RESULTS AND DISCUSSION

The development of blood leukocyte culture techniques has aided in the investigation of chromosomal anomalies in humans and other species. Formerly established techniques have proven to be inconsistent and unreliable for cytogenetic studies in cattle. For the purpose of analyzing the chromosomal complement in a herd of cattle, a reliable and consistent technique was established from modification of earlier procedures.

In modification of previous techniques (Moorhead et al., 1960), blood leukocyte cultures failed to produce adequate leukocyte growth. After reviewing favorable results in the blood leukocyte culture method of H. O. Dunn (personal communication), 0.7 ml. of 7.5% sodium bicarbonate was added to the formulated culture medium consisting of tissue culture medium 199, fetal calf serum, penicillin and phytohemagglutinin. The addition of sodium bicarbonate to the culture medium aided in maintaining a neutral pH, decreased contamination and resulted in optimum leukocyte growth.

Further improvement in the technique included a hypotonic treatment of 0.9% sodium citrate. Hypotonic

treatment of 0.9% sodium citrate was found to increase permeability of the cell without damage or breakage. Adequate results in hypotonic treatment of 0.9% sodium citrate were also reported by Bhambhani and Kuspira (1969).

Favorable results were obtained in one-hour fixations of methanol-glacial acetic acid (3:1) and an additional 15-minute fixation using 45% glacial acetic acid and 55% distilled water. The fixation period increases cell separation and the results show an increase of metaphase spreading.

The air-drying method was successfully used in slide presentation, as supported by Rothfels and Siminovitch (1958). This method of slide preparation provided a maximum number of well spread metaphases. It was observed in this study that by tilting the hot-place in slide presentation there is an increase in clear, well spread metaphases present.

This technique in blood leukocyte culture was used successfully in analyzing chromosomal abnormalities present in cattle. The chromosome complement in cattle consists of 29 pairs of autosomes (acrocentric) and one pair of sex chromosomes (metacentric), as described in Figure 1. All cows studied but one, expressed a normal chromosome complement of 60. One cow (#37) exhibited an abnormal chromosome number of 58, as viewed in Figure 2. This cow had previously given birth to a normal bull calf. The bull calf

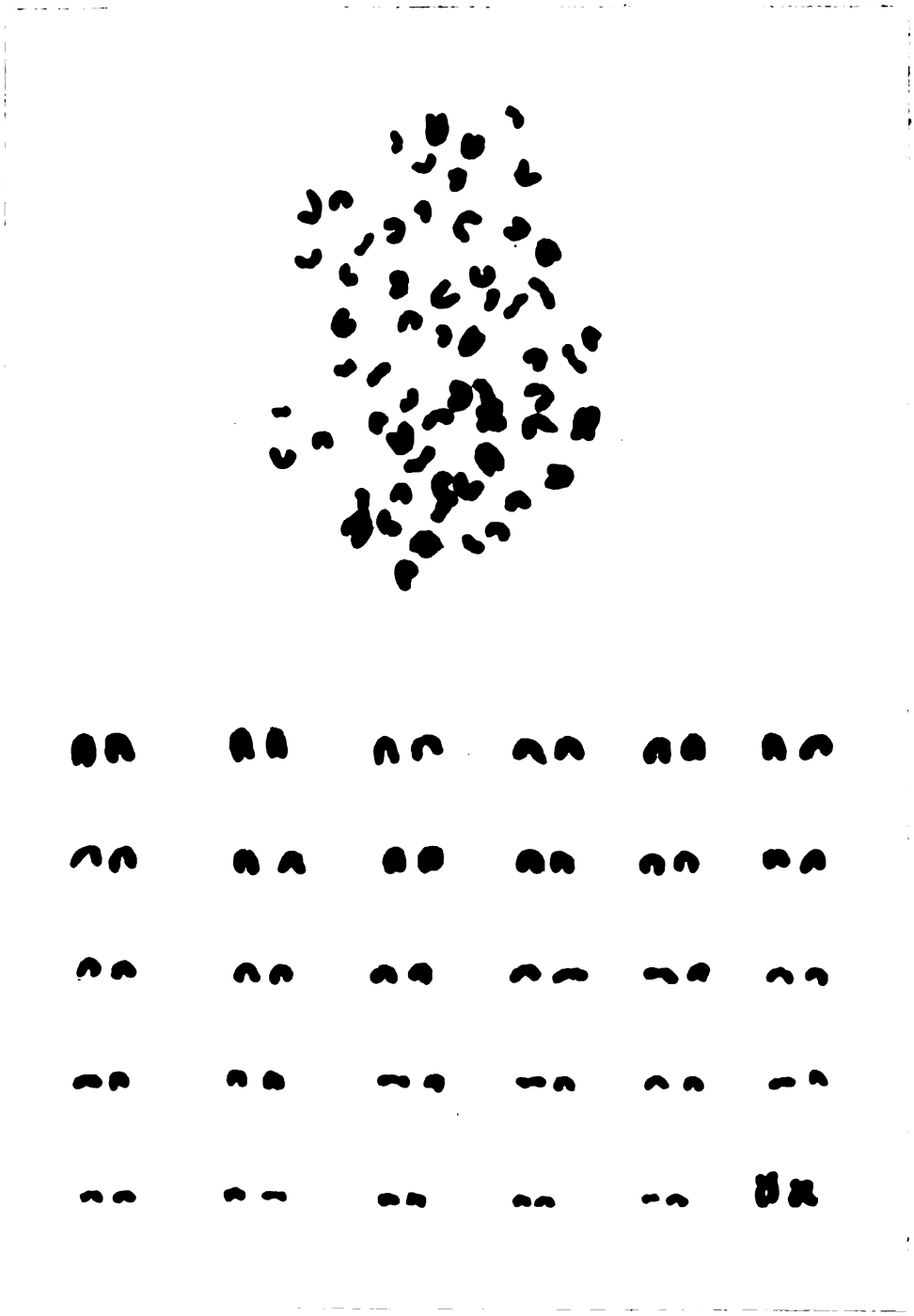

Figure 1. Karyotype of a normal female bovine, 60, XX (magnified 3000X).

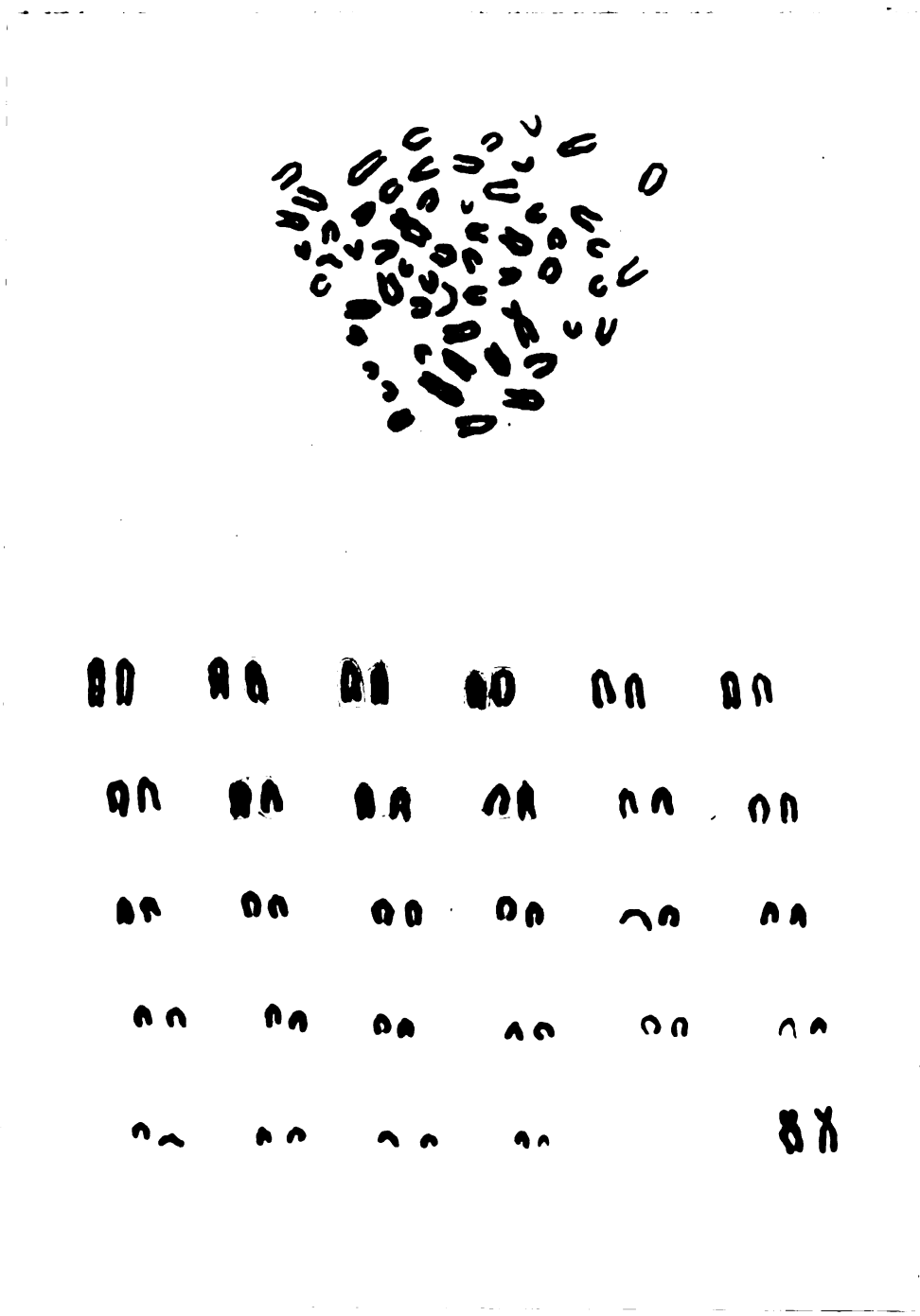

Figure 2. Karyotype of an abnormal female bovine, 58, XX (magnified 3000X).

showed no chromosomal abnormality when examined (Figure 3). The morphological difference of the X and Y chromosome between the male and female were also observed in Figures 1 and 3, the X chromosome being three to five times larger than the Y sex chromosome complement present in the male (Bhambhani and Kuspira, 1969).

Cytogenetic studies were also performed on two cows from Premier Corporation (Fowlerville, Michigan) and one cow from the drylot research project. These cows had shown a past history of producing offspring exhibiting phenotypic abnormalities. When examined, no abnormality in their chromosomal complement was found. The fertility of these cows examined was not affected during the next breeding season.

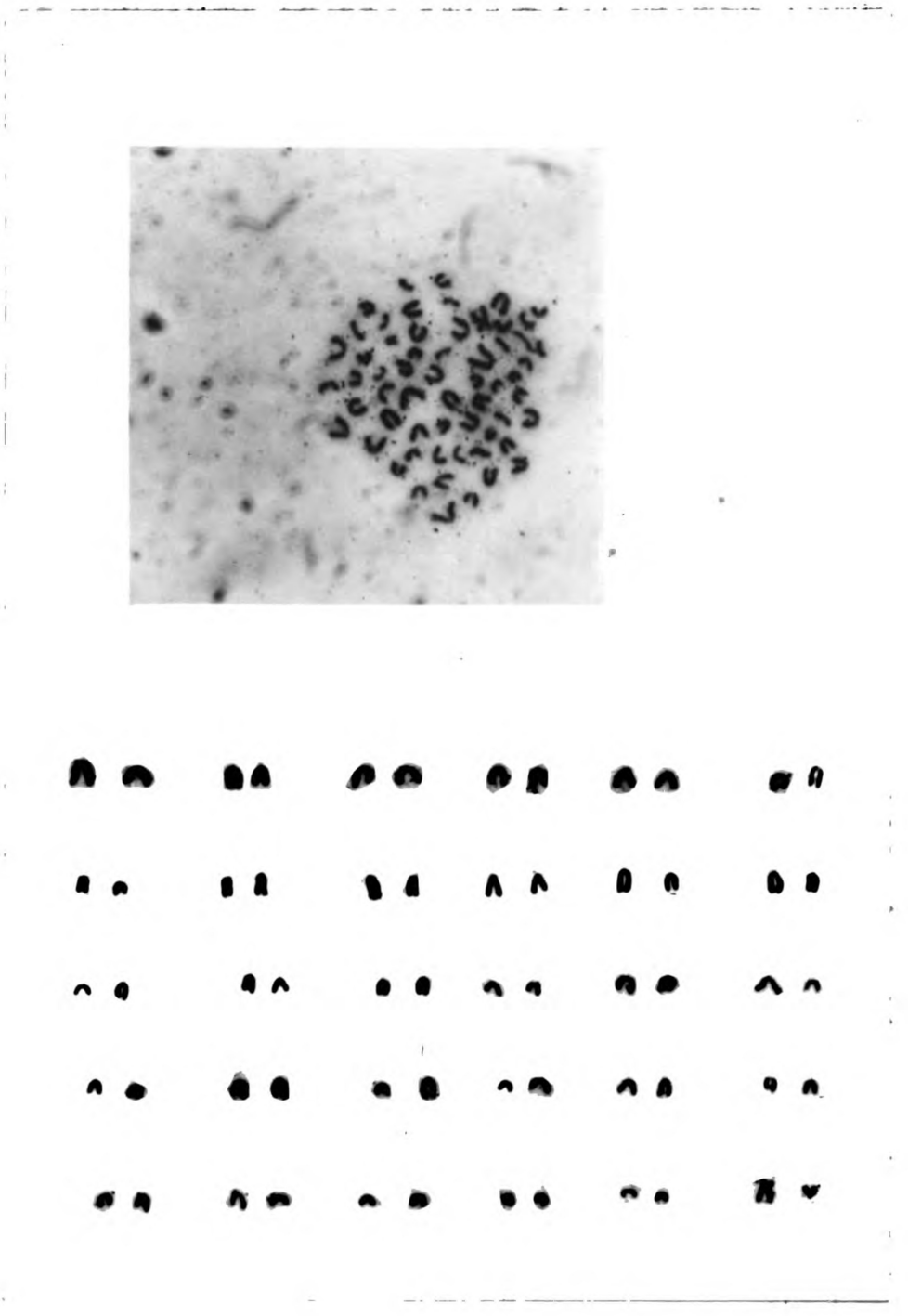

Figure 3. Karyotype of a normal male bovine, 60, XY (magnified 3000X).

SUMMARY AND CONCLUSIONS

A reliable and consistent technique was developed by modification of procedure reported by previous investigators for cytogenetic studies in cattle. This technique was used in the examination of chromosomal abnormalities present in a herd of cattle. In addition, this study was designed to observe the relationship of chromosomal abnormalities between the dam and offspring.

The following conclusions resulted from the data:

1. Hypotonic treatment of 0.9% sodium citrate increases cell permeability without cell damage.
2. Prolonged fixations of methanol-glacial acetic acid (3:1) facilitates cell separation.
3. Additional fixation of 45% glacial acetic acid aids in cell separation and increases the spreading of metaphases.
4. Air drying on a tilted hot plate is necessary for optimum slide presentation.
5. The occurrence of abnormal chromosome complements in cattle is low.

PART II

ANALYSIS OF BREEDING RECORDS IN BEEF CATTLE

INTRODUCTION

Reproductive performance in beef cattle may be maximized by observation of breeding records. Individual records can be recorded efficiently by daily observation and processing by computer. Computer analysis of breeding records can be used efficiently as a management tool in monitoring the herd's reproductive performance and fertility problems.

Fertility problems are numerous in cattle herds. Previous research has reviewed the importance of maximum reproductive efficiency. Further studies are needed in the analysis of breeding records on individuals within herds. By analyzing various parameters showing problems of infertility, reproductive performance may be enhanced.

The objectives of this investigation were:

1. To analyze breeding records on individual cows in two herds.
2. To relate various parameters to reproductive performance.

LITERATURE REVIEW

Boyd (1972) developed a computer program for recording and analyzing breeding records on individual cows within the herd. Processing and summation of data by computer printouts reveal a herd's current reproductive status. The computer printout presented numerous parameters including a list of cows in order of calving, conception rates by bulls, services per conception, age of dam, interval from calving to first service, interval from first service to conception, calving interval and calving difficulty.

Britt and Ulberg (1969) observed the reproductive performance of seven dairy herds. The factors analyzed included services per conception and average days open for cows exhibiting low fertility levels. There was a decrease in the average days open with an increase in the number of cows in the herd. This phenomenon was due to improved management in large herds through use of a herd reproductive status system.

Numerous factors have been shown to affect reproductive performance. Age of dam, level of nutrition, services per conception, interval from calving to first breeding and

interval from first breeding to conception, were all important factors influencing reproductive performance.

Reproductive efficiency has been found to decline with the increase of the age of the dam. Lindley et al. (1958), in a 17-year study of a Hereford herd, found that older cows showed longer intervals from calving to first breeding and from first breeding to conception. The older cows also required more services per conception.

Conflicting results were reported by Wiltbank (1970) in that young cows had a longer interval from calving to first estrus than older cows. The average interval from calving to first estrus was 53.4 days in cows 5 years or older, 60.2 days in 4-year olds, 66.8 days in 3-year olds and 91.6 days in 2-year old dams.

By 80 days post-partum, 80% of the cows over 5 years old had shown estrus compared to 68% of the 3-year old cows (Wiltbank, 1970). Results showed that 11% of the older cows and 32% of the younger cows failed to meet the optimum 12-month calving interval.

A similar study was conducted by Schilling and England (1968) in a crossbreeding project. The results showed no differences in fertility due to the age of dam.

The level of total digestible nutrients (TDN) received both before and after calving affects the reproductive performance of the cow. Post-partum estrus was

delayed when the amount of TDN was limited to one-half the requirements either before or after calving (Wiltbank et al., 1962, 1964; Dunn et al., 1969). The interval from first breeding to conception was longer in cows receiving the low energy level.

Many cows failed to show estrus during the normal breeding season when fed inadequate levels of energy (Wiltbank, 1968). Only 81% had expressed signs of estrus by 100 days after calving when receiving 7 lb. TDN per day. In comparison, 98% of the cows receiving 22 lb. per day showed signs of estrus during the 100-day period after calving.

Wiltbank et al. (1964) reported on the influence of post-partum energy intake on reproductive performance. The interval from calving to first estrus was 49 days in cows receiving 100% of the required energy level. Cows receiving 75% and 150% of their recommended level had an interval from calving to first estrus of 73 and 72 days, respectively. Low conception rates were shown in the cows receiving the recommended level of energy and having a short interval from calving to first estrus.

Early reports by Shannon et al. (1952), Warnick (1955) and Lindley et al. (1958) showed that cows bred at shorter intervals after calving required more services per conception than cows bred after longer intervals. Shannon

et al. (1952) reported that 52.5% of the cows conceived when serviced between 51 to 60 days post-partum while only 37.7% conceived when serviced 21 to 40 days post-partum. These results show that a minimum interval from calving to first insemination of 50 days was required for satisfactory fertility.

Warnick (1955) reported that during a three-year period the average interval from parturition to first estrus was 59.2 days in Angus cows and 62.7 days in Herefords. The conception rate for the first services was 74%. The age of the cow did not influence the post-partum estrus interval or fertility level.

Reports by Lindley et al. (1958) showed that cows bred at shorter intervals after calving required more services per conception than cows bred after longer intervals. Cows bred from 27 to 50 days after calving required 1.94 services per conception while those bred from 61 to 90 days required 1.38 services. This was supported by Perkins and Kidder (1963) who found that conception rates were higher for cows bred 79 days post-partum or later. The average number of days from first service to conception was 22.6 days.

MATERIALS AND METHODS

Premier Corporation

The data for this study of reproductive efficiency were taken from the breeding records of the Premier Corporation, Mahogany Farms Division, Williamston, Michigan. There were 200 purebred Angus cows analyzed in this study.

The cows were maintained in a wooded area. They received a corn silage-hay ration during the winter months and were pastured in the summer months. Cows were bred for year-round calving by artificial insemination and natural matings. The cows were bred for two heat periods by artificial insemination. Cows now pregnant within this interval were serviced by natural mating. The matings were planned in accordance with the overall breeding program.

Individual records of the cow herd were maintained by daily observations. Data recorded on each cow included the dam's number, dam's age, sire number, breeding dates, pregnancy tests, calving dates, sex and weight of calves. These data were recorded on each cow on note cards in a vertical sliding file cabinet. The cow's individual record was listed under alphabetical letters in numerical order by ownership of the cows.

Breeding records and other information on each cow were recorded on tape by a computer utilizing the Cobalt system. This system is unique for storage and printing large amounts of data and summation of various parameters in analyzing reproductive efficiency and infertility problems. The parameters summarized included age of dam, number of services per conception, interval from calving to first breeding, interval from first breeding to conception, calving interval, sex ratios and estrous cycle length.

Lake City Crossbreeding Project

Reproductive performance was analyzed by observation of breeding records of 100 crossbred cows from the Michigan State University Lake City crossbreeding project. The breeding project analyzed (Magee and Greathouse, 1971) consisted of the following four breeding groups: (1) straightbred Hereford cows mated to Hereford bulls selected at random, (2) straightbred Hereford cows mated to Hereford bulls selected on yearling weight, (3) Hereford-Angus-Charolais crossbred cows rotationally mated to Angus, Charolais and Hereford bulls selected on yearling weight, and (4) Hereford-Angus-Holstein crossbred cows rotationally mated to Angus, Holstein and Hereford bulls selected on yearling weight.

During this two-year analysis (1971 and 1972) of reproductive performance, the cows and heifers were

maintained on nutritional studies in a drylot situation during the winter and spring months (Greathouse et al, 1971, 1972). Nutritional levels for the cows and first calf heifers were varied to meet their proper nutritive requirements. The feeding regime in the 1971 study consisted of (1) alfalfa-grass silage, and (2) Pro-Sil (Ruminant Nitrogen Products Co., Adrian, Mich.) treated corn silage. Pro-Sil is a supplement composed of a suspension of molasses, ammonia and minerals. Cows on the alfalfa-grass silage received 19.6 lb. dry matter per head daily and lost an average of 39 lb. of body weight during the 83-day trial. The ration consisting of 16.2 lb. of dry matter from corn silage produced an average daily gain of 0.97 pounds and was consequently more than adequate for maintaining body weight.

Heifers receiving 17.4 lb. of dry matter from corn silage gained equally as well as heifers receiving 19.1 lb. dry matter from corn silage and grass-legume hay. Daily gains for both groups averaged 1.75 pounds of body weight.

Cows studied for reproductive performance in 1972 were maintained on a nutritional study consisting of: (1) corn silage treated with Pro-Sil, and (2) alfalfa-grass silage treated with Pro-Sil. Cows receiving 16.2 lb. dry matter corn silage per head daily produced an average daily gain of 1.03 lb. for the 70-day wintering period. All cows

were in above-average condition with individual gains of from 75 to 165 lb. of body weight. The ration of 19.6 lb. of dry matter alfalfa-grass silage per head daily produced a relatively poor performance of 0.01 lb. average daily gain.

In this crossbreeding project, the breeding program consists of both artificial insemination and natural matings. Replacement heifers are mated by artificial insemination for 75 days from the period of March 15 to June 1. The cows are mated for a period of 60 days, beginning April 1, by artificial insemination. On June 1 bulls are used in natural service for 30 days on both cows and heifers. Cows are maintained in a drylot situation from November 1 until June 1, and on pasture during the summer and fall months. Cows and heifers found not pregnant in the fall are culled from the breeding project.

Reproductive performance of this crossbreeding project was analyzed from individual breeding records. The individual records maintained by the herdsman were transferred to computer-data information cards. The data cards were sorted on the IBM 082 for cows that had previously given birth to at least two calves. A printout sheet of the cows was then prepared by the IBM 407 printer.

The following information in the appropriate columns had been previously collected on data cards and listed on the printer.

Card Column	Information
11-14	Cow number
15-16	Dam's age
29-34	Date last bred
36-37	Calf birth weight
41-44	Calf number
45	Sex of calf
48	Date calved
49-54	A.I. service date
55-57	Sire number
75	Times bred by A.I.

In analyzing the data, the following parameters were examined: age of dam, nutritional trials, number of services per conception, interval from calving to first breeding, interval from first breeding to conception, calving interval and sex ratio.

RESULTS AND DISCUSSION

Premier Corporation

The relationship of age of dam to services per conception and calving interval is summarized in table 1. Reproductive efficiency of the herd did not seem to be related to age of dam. Average services per conception expressed an optimum level in 14-year old dams with 1.88 services while low performance occurred in cows 13 years of age with 3.67 services. Reproductive performance has been found to decline with an increase in age of dam (Lindley et al., 1958; Bair et al., 1972).

Average calving interval in the 200 cows studied was lowest in the 8-year old dams; whereas, the 9-year old dams showed the highest calving interval of 479.1 days. The youngest dams (4-year old) had an average calving interval of 418.4 days, compared to 14-year old dams with a 448.3 day calving interval. Reproductive performance was low compared to previous investigations who reported average services per conception of 1.7 and calving interval of 12 months (Lindley et al., 1958; Schilling and England, 1968).

Younger dams had a longer interval from calving to first estrus than older dams (table 2). The average interval

TABLE 1. RELATIONSHIP OF AGE OF DAM TO SERVICES PER CONCEPTION AND CALVING INTERVAL

Age of dam (years)	No. of observations	Avg. services per conception	Avg. calving interval (days)
4	9	2.31	418.4
5	38	2.80	419.6
6	41	2.51	420.7
7	25	3.50	413.4
8	25	2.62	390.8
9	12	3.38	479.1
10	19	2.60	428.4
11	13	2.77	476.6
12	7	2.50	433.4
13	4	3.67	424.3
14	3	1.88	448.3
Avg. all ages		2.79	424.6

from calving to first estrus in 4- and 5-year old dams was 90.5 days. The 6- and 7-year old dams had an average interval from calving to first estrus of 75.8 days while 8-year olds and over had a calving interval of 75.2 days. Similar results were expressed by Wiltbank (1970) in observation by various age groups of cows at first estrus following parturition.

TABLE 2. INTERVAL FROM CALVING TO FIRST ESTRUS BY AGE GROUPS

Age of dam (years)	No. of observations	Avg. interval, calving to first estrus (days)
4 and 5	47	90.5
6 and 7	65	75.8
8 and older	83	75.2

The number of services per conception for various intervals from calving to first breeding are shown in table 3. The cows bred from 27 to 50 days after calving required 3.13 services per conception. Those bred between 51 to 60 days required 2.74 services; those bred 61 to 90 days required 2.68 services, and those bred over 90 days required 3.02 services per conception. These results show that cows serviced after 60 days post-partum are more likely to conceive, as reported by other authors (Lindley et al., 1958; Casida et al., 1968).

The reproductive perofrmance of Premier Corporation cows over the 10-year period is summarized in table 4. Calving interval is a direct reflection of reproductive efficiency for the year observed.

TABLE 3. AVERAGE NUMBER OF SERVICES PER CONCEPTION FOR VARIOUS LENGTH INTERVALS FROM CALVING TO FIRST BREEDING

Interval from calving to first breeding (days)	No. of observations	Avg. no. of services per conception
27-50	40	3.13
51-60	34	2.74
61-90	56	2.68
Over 90	65	3.02

TABLE 4. REPRODUCTIVE PERFORMANCE OF PREMIER CORPORATION

Services per conception	2.79
Interval, calving to first breeding (days)	80.9
Interval, first breeding to conception (days)	67.2
Calving interval (days)	424.6
Sex ratio (females per 100 males)	104.0

Lake City Crossbreeding Project

The relationship of age of dam to services per conception, interval from calving to first breeding and calving interval is summarized in table 5. The average services per conception showed an optimum level in 8-year old cows or older while 3- and 4-year olds showed 1.67 and 1.43 services, respectively. The average services per conception was 1.35 in this 10-year study, which is extremely high in comparison to 1.7 services reported by previous authors (Lindley et al., 1958; Hollon and Branton, 1971).

In this study, younger cows showed a shorter interval from calving to first breeding than older dams. In 3- and 4-year old dams, the interval from calving to first estrus was 53.0 and 71.7 days while 12-year old cows were serviced at 91.2 days after parturition. These results support those of Lindley et al. (1958) but Wiltbank (1970) reported that young dams had a longer interval from calving to first estrus.

The average interval from calving to first breeding for all ages was 70.5 days. Similar results were found by Warnick (1955) and Perkins and Kidder (1963).

The average calving interval in the 100 cows studied was 370.4 days. The 3-year old dams showed the most efficient production with a calving interval of 351.7 days. Reproductive performance was lowest in the 4-year old dams

TABLE 5. RELATIONSHIP OF AGE OF DAM TO SERVICES PER CONCEPTION, INTERVAL FROM CALVING TO FIRST BREEDING AND CALVING INTERVAL

Age of dam (years)	No. of observations	Avg. services per conception[a]	Avg. interval, calving to first breeding	Avg. calving interval
3	3	1.67	53.0	351.7
4	38	1.43	71.7	375.2
5	24	1.31	64.9	369.3
6	17	1.48	68.1	372.4
7[b]	..	....	....	.....
8	2	1.00	63.5	361.0
9[b]	..	....	....	.....
10	1	1.00	77.2	366.0
11	6	1.20	....[c]	367.7
12	9	1.00	91.2	359.8
Avg., all ages		1.35	70.5	370.4

[a]Services by artificial insemination, first two cycles only. Not directly comparable to services per conception in the Premier herd (tables 1 and 4).

[b]No dams present in this age group.

[c]Information not available.

with a calving interval of 375.2 days. A 365 day or less calving interval is required for optimum reproductive efficiency.

The nutritional effects on reproductive performance in the 1971 study is summarized on table 6. Each nutritional group was evaluated by services per conception, interval from calving to first breeding and calving interval. Cows in group I receiving 19.6 lb. dry matter from alfalfa-grass silage when compared to group II receiving 16.2 lb. of dry matter from corn silage, showed no differences in services per conception. Cows in group I had an interval from calving to first breeding of 65.3 days while group II returned to estrus 78.8 days after parturition. Group I showed a 13-day shorter calving interval. The interval from calving to first breeding is critical in securing a 12-month calving interval (Warnick, 1955; Perkins and Kidder, 1963).

First calf heifers in 1971 were maintained on a nutritional study and analyzed by services per conception, interval from calving to first breeding and calving interval (table 6). Heifers in group III receiving 17.4 lb. of dry matter from corn silage, were compared to those in group IV receiving 19.1 lb. of dry matter from corn silage and hay. Group IV showed 1.27 services per conception in comparison to 1.43 services per conception in group III. But group III showed a shorter interval from calving to first breeding and shorter calving interval.

TABLE 6. NUTRITIONAL EFFECTS ON REPRODUCTIVE PERFORMANCE

Nutritional group	Avg. services per conception	Avg. interval, calving to first breeding	Avg. calving interval
I	1.36	65.3	352.5
II	1.37	78.8	374.2
III	1.43	62.2	363.5
IV	1.27	76.4	378.4

Cows bred from 27 to 50 days required 1.94 services per conception while those bred from 61 to 90 days required 1.38 services. These results support previous investigators (Lindley et al., 1958; Perkins and Kidder, 1963) who reported that cows serviced at shorter intervals after calving required more services per conception than cows bred at longer intervals.

Low conception rates were also shown in cows receiving the recommended level of energy and exhibiting a shorter interval from calving to first estrus (Wiltbank et al., 1964). Cows receiving 75% and 150% of their recommended allowance had a longer interval from calving to first estrus but showed a higher conception rate at first service.

Cows receiving a high level of energy intake had an increase in milk production and longer intervals from calving to first estrus than lower energy groups (Gardner, 1969).

Carman (1955) reported that the period from parturition to first estrus was related to the level of milk production of the dam.

The reproductive performance of the Lake City crossbreeding herd is summarized in table 7. The overall reproductive performance was high in comparison to results reported by previous authors.

No 7- or 9-year old cows were present in the herd. Breeding dates were not available for summation of the interval from calving to first breeding in 11-year old dams. All breeding records observed in this study were by artificial insemination and no natural breeding dates were available.

TABLE 7. REPRODUCTIVE PERFORMANCE OF THE LAKE CITY CROSSBREEDING PROJECT

Services per conception	1.35
Interval, calving to first breeding (days)	70.5
Interval, first breeding to conception (days)	9.6
Calving interval (days)	370.4
Sex ratio (female per 100 males)	87.6

SUMMARY AND CONCLUSION

Reproductive performance was studied by the analysis of breeding records of 200 purebred Angus cows from Premier Corporation and 100 crossbred cows from the Lake City cross-breeding project. Individual information of the cow herd was recorded for computer analysis and visual observation. Reproductive performance was studied by use of the following parameters: age of dam, services per conception, interval from calving to first breeding, interval from first breeding to conception, and calving interval.

In summation, the following conclusions can be drawn:

1. Average services per conception was not related to age of dam.
2. Average calving interval was not related to age of dam.
3. Younger cows had a longer interval from calving to first estrus than older cows.
4. Cows serviced 60 days after parturition are more likely to conceive than those serviced prior to 60 days.

5. Computer analysis is an effective management tool in evaluation of reproductive performance and studying problems of infertility.

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APPENDIX

PUBLICATIONS BY AUTHOR

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Full Papers

1. Cell numbers in rabbit preimplantation blastocysts. S. Fujimoto, N. Pahlavan, H. D. Woody and W. R. Dukelow. Biol. Reprod. 1974 (in press).

2. Maximizing feeder calf production under intensive management systems progress report. H. D. Ritchie, D. A. Woodburn, R. L. Dingerson and H. D. Woody. Mich. State Univ. Farm Sci. Res. Rep. 166. No. AH-LC-713, pp. 66-70, 1972.

3. Wintering pregnant heifers in drylot on two varieties of sorghum silage and two sources of supplemental nitrogen. H. D. Ritchie, D. A. Woodburn, R. L. Dingerson, H. E. Henderson, H. D. Woody and D. R. Strohbehn. Mich. State Univ. Farm Sci. Res. Rep. 174. No. AH-BC-715, pp. 55-65, 1972.

Abstracts

1. Wintering heifers in drylot on silage and NPN. H. D. Ritchie, D. A. Woodburn, R. L. Dingerson, H. E. Henderson and H. D. Woody. J. Anim. Sci. 35:1093, 1972.

2. Cell numbers and mitotic index of the rabbit blastocyst. S. Fujimoto, H. D. Woody and W. R. Dukelow. Fed. Proc. 32:214, 1973.

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