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(Signed)..Keith Winchell....

PERMANENT ADDRESS:
Box 3957..............
Fort St. John.....
British...Columbia,. Canada.

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THE EFFECT OF HORMONE-INDUCED MULTIPLE BIRTHS
ON
NUTRITION OF HOLSTEIN COWS AND CALVES

by

© KEITH SMITH WINCHELL

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

FALL, 1972

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "The Effect of Hormone-Induced Multiple Births on Nutrition of Holstein Cows and Calves" submitted by Keith Smith Winchell in partial fulfilment of the requirements for the degree of Master of Science.

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Supervisor

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Date August 30, 1972

ABSTRACT

The Holstein breeding herd at the Dairy Research Unit was subjected to hormone treatments, based on the work of Turman et al. (1969), in an attempt to produce twin pregnancies which could be used in evaluating the energy requirements in the last part of gestation of cows producing twins and of the twin calves. Unsatisfactory results in this treatment led to several adjustments in the hormone treatments throughout the experiment. Although not originally designed to compare or evaluate various hormone treatments, a number of trends were noted and speculations made from the results obtained. None of the hormone treatments was rated as satisfactory in producing a reasonable increase in calf crop percentages. Multiple births in excess of twins were almost always born prematurely, and mortality was very high. All twins born within two weeks of term were born alive and all lived to the end of their 16-week test period.

The feed consumption and weight changes of cows was recorded in the last three months of pregnancy. The recommendation by the National Research Council (1971) of 0.21 megacalories of digestible energy daily per unit of metabolic weight, corresponds closely to the amount apparently required by cows producing singles in this experiment. The cows producing multiple offspring within several weeks of term consumed 0.25 megacalories of digestible energy per unit of metabolic weight. This level, which is slightly higher than the 0.21 megacalories of digestible energy per day per unit of metabolic weight recommended by the National Research Council (1971), was considered a satisfactory minimum for normal calving.

Birth weights of those multiples born alive and completing the 16-week test period, were significantly lower ($P < 0.01$) than those of single calves. Similarly, the multiple-born calves consumed more whole milk ($P < 0.01$), less calf meal ($P < 0.01$) and had lower final weights ($P < 0.01$) than single calves. Average daily gain was greater for single calves than for multiples but differences were not significant ($P < 0.05$). The extremely poor performance of one set of twins was responsible for a great deal of the differences noted between single calves and calves born in a multiple birth group. The performance of other multiples was satisfactory. Production per cow was greater for cows producing multiples than for cows producing singles when total calf weight at 16 weeks was used for evaluating production. Six sets of twins and two calves living from a set of triplets were included in the 'multiple' group.

From the standpoint of energy requirements, both of cows producing twins and of twin calves, it seems evident that the production of a higher proportion of twin calves, at least under management similar to that in this study, is a reasonable goal.

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INTRODUCTION

During the last decade some cattle operations have been managed much more intensively in an attempt to maximize returns. With more information available on nutrition, breeding, animal health and economics of production, producers want to use this knowledge in influencing some factors of production in their favor.

Perhaps the most widely recognized advances are taking place in the areas of genetics and reproductive physiology — specifically with respect to the cow. Since a bull can breed many cows, it is only natural that research found ways of identifying superior bulls and extended the influence of these superior bulls to even more cows. Artificial insemination has played, and is still playing, an important role in improved cattle breeding and larger profits. But if the study of semen collection, storage and use, with its associated study of male reproductive physiology provided a challenge to early workers, then the desire to extend the reproductive influence of the cow in beef improvement provides an even greater challenge. The reproductive physiology of the cow must be understood, not only through maturation and release of the egg — which might correspond to the maturation and release of sperm in the bull — but throughout the resulting fertilization of the egg, implantation and pregnancy.

The desire to extend the genetic influence of the cow has led to techniques in heat synchronization, superovulation and ova transplantation, all of which are being modified as new information becomes available. A genetically superior donor cow can be superovulated, mated, and the fertilized eggs transferred to inferior recipient cows, synchronized to the donor cow's heat period, for the duration of pregnancy. Most efforts with cattle have been directed at placing a single fertilized ovum in the recipient cow. An extension of this technique, and one which has been studied as a procedure in itself, is that of superovulation to produce multiple offspring.

An increase in cow productivity through hormone-induced twin pregnancy appears to offer potential for increasing production and profits. The Alberta Cow-Calf Enterprise Analysis (1970) reported an average calf crop weaned, on the basis of cows exposed, of 87.3 percent. A successful program of induced twin pregnancies could reduce the cost of maintaining the cow herd in relation to herd productivity if it produced a substantial increase in percentage calf crop.

With trends toward consumer acceptance of leaner meat, the dairy producer is in a position to benefit more from multiple births than is the beef producer. His herd is handled daily for milking and often for feeding. Under such circumstances, administration of hormone treatments would mean less work than it would for the beef operator, although synchronization of beef cows could alter this. Dairy calves are normally raised artifically, so that problems of the dam accepting twin calves, or of being able to feed them, are not applicable.

A successful hormone program should

(1) enable the release of a sufficient number of ova to produce a high proportion of twin pregnancies

(2) enable cows to carry twin fetuses to term through hormonal and/or nutritional manipulation, and

(3) ensure that the nutritional and/or hormonal condition of the cow is adequate for delivery of strong, healthy calves, for

milk production corresponding to her potential, and for rebreeding within a reasonable period of time.

The relationship between nutrition and reproductive hormones has been of interest to researchers. In a program designed to produce multiple births it becomes even more important. At the producer level it is usually felt that multiple offspring are unthrifty and require extra care to raise. It is possible that a realization of the nutrient requirements of cows carrying multiple fetuses, and some modification in handling of the calves, could lead to a better acceptance of multiple births and greater profits.

On the basis of a recent program of treatment at Oklahoma State University using beef cows (Turman, et al., 1969) this project was initiated to attempt to determine some of the nutritional requirements necessary for Holsteins carrying more than one fetus, and to add to the information available on hormone treatments to induce multiple births. The problems, progress, and possibilities involved in the program are discussed in the succeeding sections.

REVIEW OF LITERATURE

The Bovine Estrus Cycle

Estrus Cycle

With the onset of puberty (the age at which reproduction becomes possible), usually between the 9th and 18th month in the Holstein (Cole and Cupps, 1969), the heifer exhibits more or less rhythmic sexual activity. The total of the physiological changes involved in the rhythmic sexual cycle is usually measured by the time from one "estrus" or "heat" to the next. In dairy cattle the length of the estrus cycle varies from 18 to 24 days with a mean of 21.3 days (Cole and Cupps, 1969). Estrus is defined as that period when the cow will accept the bull and may involve such other physical manifestations as nervousness, bawling, mounting other animals, slight reddening and swelling of the vulva, and the discharge of a light mucus from the vagina.

On the basis of the physiological changes which take place, the estrus cycle has been divided by most authors (Lasley, 1968; Dutt , 1966; Rice et al., 1967) into 4 phases—each phase merging into the next. The 4 phases and the levels of the 4 main reproductive hormones responsible for these phases are shown in Figure 1. Proestrus is the phase where secretory activity of the corpus luteum (a gland formed in the ovary at the site of the last ruptured follicle) is declining, allowing follicle stimulating hormone (FSH) from the anterior pituitary to activate the ovary in the development of a follicle. Estrogen activity is increasing. In the cow this period lasts from 3-5 days. Proestrus is followed by estrus. During this period estrogen activity is at its peak, bringing about the physical symptoms of estrus, also known as 'heat'. The period of estrus lasts from 6-18 hours with an average

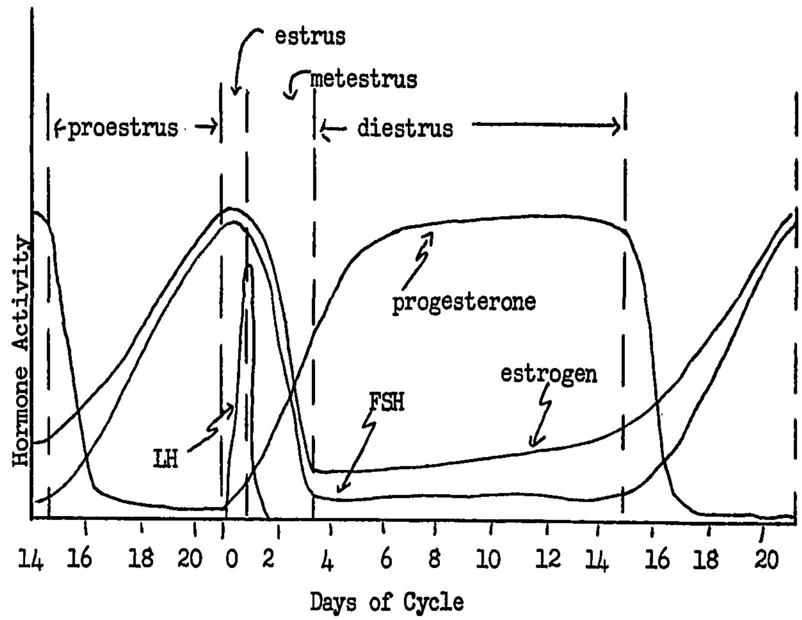

FIGURE 1. SCHEMATIC ILLUSTRATION OF THE ESTRUS CYCLE OF THE COW. (The diagram shows the four phases of the estrus cycle and gives an illustration as to the times of peak activity of each of four reproductive hormones. No attempt has been made to place a unit on the levels of the hormones; the amount of any one hormone at any one point does not necessarily correspond to the amount of another hormone at that point.)

of 12-16 hours. Estrus is followed by metestrus. During metestrus, estrogen activity declines and luteinizing hormone (LH) from the anterior pituitary causes the mature follicle to rupture (ovulation) about 12-14 hours after the end of heat (Johansson and Rendel, 1968). The ruptured follicle is re-organized to form a corpus luteum, and begins its secretion of progesterone. This period lasts from 1-3 days. Following this is a relatively long diestrus period of 14-15 days when the progesterone of the corpus luteum exerts its influence over the system. Estrogen secretions also increase slightly during this period. If the egg which was released is fertilized, then the corpus luteum remains functional throughout pregnancy and a period of anestrus results. If the egg is not fertilized then the corpus luteum regresses after an effective life of 15-16 days and the cycle begins again. Descriptions of the bovine estrus cycle in greater detail are available from a number of sources (Johansson and Rendel, 1968; Asdell, 1968; Yeates, 1965).

The estrus cycle of the cow is not an entity in itself but part of the physiology of the whole animal. As such, an animal's productivity is under genetic, hormonal, health, environmental and nutritional influences.

Genetic and Hormonal Influence

The development of breeds or lines within breeds which differ in prolificacy is evidence that fertility may have a genetic basis (Rice *et al.*, 1967). Genetic factors affecting reproduction range from those altering the structure of the reproductive tract through to those controlling, in some manner, the reproductive hormone balance. In the latter case, environmental and nutritional influence may interact with genetic capacity to the extent that isolation of intrinsic genetic factors may be impossible. Foote (1970) discusses some of the physiological reproductive defects of a hereditary nature such as hypoplasia of the gonads, defective gamete formation, silent heat, and cystic ovaries, in his paper on the inheritance of fertility.

According to Morrow (1970), infertility losses in high-producing dairy herds are caused by retained placenta, metritis, anestrus, silent estrus, cystic follicles, repeat breedings and abortion. Cystic follicles and silent estrus are under hormonal influence.

It was generally felt that follicular cysts resulted when the level of FSH was adequate for the development of a follicle or a number of follicles but LH levels were not sufficient to cause ovulation. Under these circumstances estrogen levels are high due to the presence of a mature follicle or follicles causing animals to display heat symptoms with some frequency, a condition known as nymphomania. There is some indication, however, that the cystic condition of the ovaries may result from an endocrine upset not of ovarian origin (Nalbandov, 1964). The condition may correct itself; however, if it does not do so within a short period, or if help is not given in the form of exogenous LH, then the condition may result in other body changes, primarily those of masculinization. Since it is generally felt that in the bovine LH supplies are normally in excess (Gordon et al., 1962), nymphomania as a result of cystic follicles should not be too common if only the ovaries are involved. Nalbandov (1964) reports cystic ovaries to be much commoner in dairy cattle than in most beef herds.

Liptrap (1970) divides follicular cysts into two types: the first type corresponds to those which cause extended and/or frequent heat periods with the resultant physical changes in the cow. He cites Yamauchi et al., (1954) as finding that these cysts develop under an improper FSH to LH ratio. This would correspond to Nalbandov's (1964) explanation that such cysts may develop from an endocrine upset not of ovarian origin. Liptrap's second type of follicular cyst is one which has developed under the proper FSH to LH ratio but with insufficient LH to cause ovulation. Such cows show only a mild estrus or no estrus at all. Treatment with LH activity is usually effective. The persistent corpus luteum which

Liptrap discusses is frequently caused by uterine pathology (Lynn et al., 1966) preventing a luteolytic substance produced by the uterus from initiating a regression of the corpus luteum. The persistent corpus luteum may be manually expressed per rectum thus allowing a new follicle to mature under the influence of FSH. The exact cause of cystic follicles or the persistent corpus luteum is individual and, in most cases, goes unexplained.

'Silent heat' is the occurrence of ovulation following normal, or usually normal, cyclic activity except that the symptoms of estrus do not occur or are undetectable. Gordon et al. (1962) discuss the normal incidence of silent heat and noted that the condition tends to increase calving intervals in herds where cows are artificially inseminated, since heat periods go unnoticed and cows are not bred.

Environmental and Health Influence

Cattle are a polyestrus species, showing cyclic activity throughout the year, unlike the mare which responds only during a time of increasing daylight or sheep and goats which respond during decreasing daylight (Hammond, 1955).

The estrus cycle may be affected by low temperatures accompanied by poor feeding conditions (Hammond, 1961). Dale et al. (1959) found puberty to be delayed in heifers maintained at 80 F compared to those maintained at 50 F. Under adequate feeding regimes, the normal temperature ranges usually are not considered to have any marked effect on the estrus cycle, although most authors (Yeates, 1965; Lasley, 1968) recognized some variation in breeding efficiency from season to season.

Disease factors which normally affect the estrus cycle are those which cause uterine infection. Specifically if the infection causes a retained placenta, normal cycling may be delayed. Low level uterine infection usually permits normal cyclic activity; however, implantation in the infected uterus is not successful.

Nutritional Influence

Early work of Durrell (1951) suggested that malnutrition was believed to be a direct or indirect cause in almost half the cows which showed sterility. Researchers Hignett (1950) and Hignett and Hignett, (1951) were concerned with the role of calcium and phosphorus and of Vitamin D in cattle fertility. Boyd (1970b) referred to the National Academy of Science Committee Report (1968) which found that lack of sufficient energy is one of the most common forms of nutritional infertility. Also mentioned is the fact that limited phosphorus intake is probably the most prevalent mineral deficiency affecting reproduction. It is probably safe to say that, in general, if the nutrient requirements of the cow are being met to satisfy her other needs of general health and production, then the nutritive requirements for reproductive cyclic activity should be met.

Natural Multiple Births

Of concern in studying techniques and possibilities of superovulation, is the natural occurrence of multiple births. A knowledge of natural multiple births may lead to an understanding of the types of animals and/or types of environment which lend themselves to multiple births.

Gordon et al. (1962) surveyed literature to determine the percentage of natural twins. Results ranged from 0.22 to 9.95 percent with most figures being between 1 and 3 percent. Bowman et al. (1970) did a survey to get information of heritability of twinning. Their incidence of twinning for bull progeny groups ranged from 0 up to 8.8 percent. They reported an average twinning incidence of 2.8% when information on first calvings was omitted. The twinning percentage in herds of Holstein cows has been shown to be 3.95 by Pfau et al. (1948), 2.91 by Meadows and Lush (1957), 4.2 by Erb et al. (1960), 3.03 by Hewitt (1934), and 4.50 by Erb et al. (1959). Hendy and Bowman (1970) found that the percentage of twin calvings was consistently lower in beef herds than in dairy herds. Gordon et al. (1962) draws attention to the fact that the occurrence of natural multiple ovulations has been estimated to vary from 4.1 to 13.1%, somewhat above the percentage twins born.

Hendy and Bowman (1970) surveyed the occurrence of twin births, their effect on future cow productivity and the performance of twin-born calves. The percent twins appeared to increase with the age of the dam in most studies. They cited a number of researchers as showing an increase up to the 10th to 13th year followed by a very gradual decline or a constant twinning percentage.

Seasonal variation in twinning percentage has been noted in a number of studies (Johansson, 1932; Vainikainen, 1946; Frey, 1959) (cited by Hendy and Bowman, 1970); however, peak seasons of twinning appeared to vary with the study, leaving one to speculate on the importance of seasonal variation as a general rule. Hewitt (1934) found no seasonal variation.

Gestation lengths of twin pregnancies have been shown by a number of workers to range from 1.5 to 10 days shorter than single pregnancies (Hendy and Bowman, 1970). These authors speculated that "the decreased gestation period may be due to the increased total weight carried by the dam with twin calves and the greater demand on nutrition made by the twins during pregnancy".

Calving difficulties with twin births are usually restricted to an increase in problems of presentation (both calves coming at once) and an increase in the incidence of retained placentae following calving. Hendy and Bowman (1970) cite Comberg and Velten (1962) as finding a 40% increase in retained afterbirth in dams of twins when compared with dams of singles. Although many authors feel there is a greater number of calving difficulties with multiple births, Hammond (1959) and Gordon et al. (1962) both felt that cattle, carrying twin fetuses, who received extra feed in the last part of pregnancy had fewer difficulties than expected.

Breeding efficiency following twin pregnancy is generally felt to be poorer than that following single pregnancies. Korkman (1947) (cited by Hendy and Bowman, 1970) reported an increase of 3.5 times in sterility after twin calvings compared with single calves. Pfau et al. (1948) found in a Holstein herd that, after twinning, 38.46% of the cows continued to reproduce normally (except for poorer conception rates and longer calving intervals), 12.85% became sterile, 30.77% were sold for various reasons and 7.69% died after the twin birth.

Milk production of dams producing twins has been variable compared to their production following a single birth. Hewitt (1934) and Dabash (1964) and others(Hendy and Bowman, 1970) reported an increase in milk production after a twin birth. Depressions in the milk yields of dams of twins have been noted by Meadows and Lush (1957) as well as in a number of papers reviewed by Hendy and Bowman

(1970).

The mortality rate of single calves was estimated by Dabash (1964) to be 5.72% compared to 18.79% for twin born calves, while Hendy and Bowman (1970) cite Johansson (1932) and Richter (1955) as finding mortality rates of 4.2 and 3.7% respectively for singles and 12.6 and 5.1% respectively for twins.

Birth weight of twin-born calves in the papers reported by Hendy and Bowman (1970) generally ranged from 70-80% of the weight of a single-born calf.

Erb and Morrison (1959) report that only 9% of female calves born co-twin with a bull are fertile. The freemartin condition is discussed by Nalbandov (1964) and by Gordon et al. (1962). Other than the high incidence of freemartinism in females born co-twin to a bull, most researchers feel that beyond lower birth weights and possible lowered vitality early in life (Gordon et al., 1962), the performance of twins is comparable to that of singles.

Artificial Control of the Estrus Cycle

The desire for heat synchronization (or suppression), superovulation, and ova transfer have led to the development of various techniques to achieve these ends.

Work in heat suppression or synchronization has generally centered around the use of progesterone or some derivatives of it which have the same action — namely, the inhibition of FSH from the anterior pituitary. Ginther (1970) found that progesterone given for short periods starting up to 2 days post-estrus shortened the length of the estrus cycle while injections of progesterone starting on day 8 increased cycle length. Roussel and Beatty (1969) fed

MGA[1] in synchronizing dairy cows and found a mean interval from withdrawal to first estrus of 4.9 days after 14 days of MGA. Boyd (1970a) and DeBois and Bierschwal (1970) used progestinated intravaginal pessaries with some success. Other researchers in the area of heat synchronization have been Laing and Fosgate (1970, 1971), and Lamond et al., (1971).

Superovulation has been brought about in a number of species for the purpose of transferring the fertilized ova to recipient dams. Dowling (1949) successfully transferred ova in rabbits. Ova transplantation has also been successfully performed in sheep by Warwick and Berry (1949) and Karihaloo (1970), in pigs by Webel et al., (1970), and in cattle by Willet et al. (1951), and more recently on a limited commercial basis (Focus on Beef, September, 1971; June, 1972).

Superovulation

The exact amounts and times of FSH and LH released into the cow's system from the anterior pituitary during the estrus cycle are not known but probably vary considerably from cow to cow depending on the hormone balance. Whatever those levels and times are, however, they are normally adequate for the complete maturation and rupture of a single follicle. Most researchers feel that endogenous LH is secreted in abundance in the cow (Gordon et al., 1962; Hammond, 1955) and is adequate for the rupture of more than one follicle if more than one follicle is mature. The amount of FSH then, is limiting to a single follicle. If more than the normal amount of FSH acts on

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[1] Registered Trademark, The Upjohn Company, Kalamazoo, Mich.

the ovary, then more than one follicle will develop. The number of extra follicles which develop will depend on the level of exogenous FSH activity introduced.

Various animals, such as the pig, mare and ewe (Foote and Onuma, 1970), have all been sources for pituitary hormone — both FSH and LH fractions.

These extracts have been used both homologously (extract from species A used on species A) and heterologously (extract from species A used on species B) with varying results. Work on the influence of FSH led to the discovery that blood serum of pregnant mares (PMS) contained a significant amount of FSH activity (Cole and Hart, 1930). Since PMS is a more readily available source of FSH activity than anterior pituitary extract it has become the main source of FSH in superovulation work.

A majority of researchers report the occurrence of refractoriness (a decrease in response with repeated use) with the use of PMS and FSH fractionsof anterior pituitary extract. This was attributed to antibody formation against the protein extract from a different species source. Hafez et al. (1964) and Willet et al. (1953) found refractoriness in cows, and Pigon et al. (1960) reported refractoriness in sheep. Refractoriness to horse anterior pituitary extract was noted in rabbits by Maurer et al. (1968) and Adams (1961). Where refractoriness was reported it appeared to be specific; for example, cows refractory to PMS injections would respond to anterior pituitary extract from pigs (Hafez et al., 1964). Where refractoriness to gonadotropins was reported, time failed to reduce the degree of refractoriness (Willet et al., 1953), or where some reduction in refractoriness was noted, it was regained very rapidly on resumption of treatment with gonadotropins (Pigon et al., 1960). Cole et al. (1957) concluded that "antihormone formation is not a serious factor in the clinical use of PMS in cattle if minimal physiological doses are employed". Gordon et al. (1962) reported an anaphylactic reaction in one out of 525 cows injected.

Although gonadotropins, and more specifically PMS preparations, have been successful in increasing multiple births in a number of species, response in the cow has been variable. Since Casida et al. (1934)(cited by Foote and Onuma, 1970) first superovulated a cow, a number of researchers have used various PMS preparations in varying dosages at various stages of the estrous cycle in attempts to get a more consistent response. Most recent superovulation attempts have been made in the absence of progesterone influence, either by timing the administration of PMS to coincide with the beginning of the normal follicular phase (days 15 to 17 or 18)(Gordon et al., 1962; Scanlon, 1972), or by the manual expression of the existing corpus luteum to remove the influence of progesterone and thereby create a follicular phase (Hafez et al., 1964).

The levels of PMS used in superovulating cattle have varied and are complicated to some extent by the fact that different forms of PMS appear to have different responses although biological assay with rats has revealed the same potency (Hafez, 1969). Brock and Rowson (1952) reported a descending order of response in cows when whole, freeze-dried and purified PMS preparations were compared. Confusion also exists in that some dosage levels are reported in millilitres of serum or in "Rat Units" rather than "International Units" (Gordon et al., 1962). Cole and Erway (1941) report the

"Rat Unit" to be equal to the "International Unit". Another point which makes comparison of research efforts difficult is the handling of animals under study. Depending upon the objective of the author some animals are slaughtered soon after treatment to examine the ovulatory response (Hafez et al., 1964), some animals are not slaughtered but undergo extensive and/or repeated physical examinations to determine ovulatory response, embryo implantation and fetal development (Gordon et al., 1962), and some animals are left after treatment letting the result of the gestational period serve as an indication of the degree of response to the treatment (Turman et al., 1971). While each of these are important in elucidating the total response of the cow to exogenous FSH activity, comparison of results, where made, must take into account the methods of measuring the response.

A number of PMS levels are employed and the responses obtained are shown in Table 1. The results give only the number of ovulations; they give no indication of follicles which did not rupture nor in any way do they illustrate the manner in which the hormone treatment was carried out.

The work of these researchers and others would indicate that response increases with dosage level. Gordon et al. (1962) report, however, that variability in response also increases with dosage level. Very high doses of PMS led to many unovulated follicles (Dowling, 1949; Willet et al., 1952) and a level of 5000 IU was reported by Hafez et al. (1963) to cause hemorrhagic follicles. Some animals show no superovulatory response even after large doses of PMS (Hafez, 1969). Hafez (1969) lists some of the reasons for

TABLE 1. Response to PMS injections

Reference	IU of FSH activity	Mean No. of ovulations
Gordon *et al.* (1962)	800	1.43
Gordon *et al.* (1962)	1,000	1.77
Gordon *et al.* (1962)	1,200	2.50
Hafez *et al.* (1964)	1,500	2.4
Gordon *et al.* (1962)	1,600	2.71
Scanlon (1972) (cycle following 3,000 IU dose)	2,000	19.5 (± 7.80)
Gordon *et al.* (1962)	2,000	3.97
Scanlon (1972) (cycle following 2,000 IU dose)	3,000	10.7 (± 4.72)[a]
Hafez *et al.* (1963) (cited by Foote and Onuma (1970)	3,000	33 (range 14-104)
Scanlon *et al.* (1968)	3,000	9
Hafez *et al.* (1963) (cited by Foote and Onuma (1970)	4,000-7,000	≃12 (range 2 — 28)

[a] Mean ovulation rates of 5 ovulated cows (2 cows did not ovulate) were 15.0 (± 5.54)

variability of response as season of the year, breed, liveweight, stage of the estrus cycle at injection, individual variations in cycle length, the post-partum interval, and plane of nutrition. Other reasons include age (Zarrow and Wilson, 1961; Fox *et al.*, 1964), genetic constitution (McLaren, 1962; Lin and Bailey, 1965), and the amount of hormone administered previously (Edwards and Fowler, 1958; Lin and Bailey, 1965).

Although LH activity in the cow is considered adequate for ovulation of more than one follicle a number of workers have injected

LH active substances at estrous following FSH stimulation of the ovary to ensure that all developed follicles are ovulated. Human chorionic gonadotropin (HCG), from the chorionic membrane of the human placenta and excreted in the urine of pregnant women, is the substance of choice for LH activity in most recent research.

After the problems of ovulation in response to FSH stimulation are solved come those of fertilization, implantation, and maintenance of pregnancy to term. Hafez (1969) reports a generally lower fertilization rate in superovulated farm animals, due partly to lack of sperm transport, partly to lack of sperm capacitation, partly to reduced egg recovery by the infundibulum, and partly to rapid oviductal transport under the influence of several corpora lutea.

The ability of the cow to carry out implantation and sustain pregnancy is under the influence of a number of factors. One of these is the amount of progesterone secreted by the corpora lutea. Hafez (1969) reports that the total amount of luteal progesterone is higher in cows with multiple corpora lutea than in singly ovulating cows (Hafez _et al._, 1965) although the amount of progesterone per corpus luteum is less. In an attempt to determine the ability of heifers to carry twin offspring to term, Rowson _et al._ (1969) transferred 2 fertilized ova into each of 16 heifers. Only 2 (12.5%) produced twins at term, the remainder producing singles. This would indicate that more than the availability of 2 fertilized ova may be necessary for multiple births. Other factors governing the maintenance of pregnancy include the distribution of embryos between uterine horns, the spacing between embryos in the same horn, genetic capacity of the cow, environmental factors, such as temperature and possibly season, and the nutrition of the cow.

Nutrition and Multiple Births

Research has been done on the nutrient requirements for pregnancy in cattle; however, research on the nutrient requirements for twin pregnancies is very limited. Gordon *et al.* (1962), Hammond (1959) and Turman *et al.* (1971) all felt that an increased level of nutrition in the last part of pregnancy gave good results with twin births. Precise levels of nutrients were not discussed in these experiments so the amounts of energy and protein recommended above the levels indicated in National Research Council (NRC) requirements (1971) for single pregnancies need further clarification.

Nutrition cannot be separated completely when discussing the factors responsible for enabling a cow to maintain a multiple pregnancy to term. However, it is one factor which can be controlled and in so doing determine whether proper nutrition will increase the number of animals capable of producing live multiple births. Adams (1953)(cited by Hammond, 1955) found a response at an earlier age in rabbits on a high plane of nutrition and Marden (1953) found no response in poorly nourished calves.

Gardner (1969b) fed two groups of Holsteins at 115% and 160% of digestible energy requirements for maintenance based on NRC (1958) recommendations during the last 6 to 8 weeks of gestation. From his work he states: "Although the number of twin births was few, the birth weights of these calves were obviously not reduced by feeding dams 115% of their maintenance energy requirement". (This work did not involve hormone treatment to induce multiple births.) Hight (1966) found that calf birth weight and vigor are partially attributable to levels of energy offered during gestation, particularly during the last month before parturition. In a previous paper on the same experiment, Gardner (1969a) suggests that daily NRC recommendations for the actual needs of pregnancy are apparently 6 to 10 megacalories (Mcal) of metabolizible energy too high.

EXPERIMENTS AT THE UNIVERSITY OF ALBERTA

Introduction

The objective of this study was to examine some nutritional requirements of cows carrying more than one calf and of the calves that were born. Holstein cows were subjected to a number of hormone treatments in an attempt to gain information on the effectiveness of the treatments in promoting multiple births, but primarily in an attempt to produce enough multiple births so that information could be obtained on the nutritional requirements of multiparous cows and their calves. Feed intake of cows was recorded during the last twelve weeks of gestation during which time an attempt was made to regulate weight gain to correspond to the estimated requirements of developing twin fetuses. Feed consumption and weight gains of calves born were recorded to four months of age. Digestibility trials, using four lambs, were carried out using all rations consumed by all groups of animals.

Experimental

Hormone Treatments

The hormone treatments were based on a method used by Turman et al. (1969) at Oklahoma State University which resulted in 44% multiple births in treated beef cows on the basis of animals conceiving. As the experiment progressed it became evident that the hormone treatment would have to be altered since the animals were not reacting as the animals in the Oklahoma study had done; abortions of multiple fetuses were quite common and a number of cows returned to heat after varying lengths of time without having shown any signs of aborting. As a result of attempting to use a more successful hormone level and because of the continuous nature of the breeding program, the number of animals in each treatment group varies. Data of conception rate to first service, the number of services to conception, breeding interval, abnormal estrus cycles, silent heat, and the number of calves born were recorded and compared on the basis of hormone treatment.

Trial 1A

The first heat period after cows and heifers became available was recorded as day 0 of the estrus cycle. On day 5 of the cycle each animal was given an intramuscular (IM) hip injection of 1500 International Units (IU) of pregnant mare serum gonadotropin (PMS)[1]. On day 16 each animal was given a second IM injection of 2000 IU of PMS. On day 21 or whenever the cow showed heat, whichever came first, the cow was given an intravenous (IV) injection (jugular vein for heifers, mammary vein for milking cows) of 2500 IU of human chorionic gonadotropin (HCG)[2] and bred within 8 hours of the injection. Cows which showed heat 1-10 days after being bred were re-injected with HCG and rebred.

Trial 1B

Before the experiment was started it was anticipated that dairy cattle might respond more to a given hormone level than beef cattle, based on the fact that twinning is naturally more prevalent in dairy cattle. Based on this premise and the desire to avoid refractoriness

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[1]Equinex, Ayerst Laboratories

[2]APL, Ayerst Laboratories

to high levels of PMS if lower levels would produce a response, at least initially, cows which did not conceive in trial 1A were subsequently treated with a lower level of PMS.

Cows which returned to heat 11 days to 6 weeks after being bred in trial 1A were given an IM injection of 1000 IU of PMS on day 5 following this estrus and 1500 IU of PMS on day 16. They were also given an IV injection of 2500 IU of HCG on day 21 or whenever they showed heat, whichever came first, and were bred. If the cow again returned to heat 11 days to 6 weeks following the second breeding, this regime of treatment was again followed. Most animals which did not settle to this third breeding were no longer treated but bred each heat period until they conceived or until they were sold because they did not conceive. Trials 1A and 1B were followed until initial results indicated that the levels used for the first treatment breeding, i.e. 1500 IU of PMS on day 5 and 2000 IU of PMS on day 16, were too high and were causing abortions. At this point treatments were modified to bring levels of all cows treated into line with those of trial 1B.

Trial 2

The modifications initiated in the treatment were:

(1) All animals received an IM injection of 1500 IU of PMS on day 16 only (i.e. no PMS on day 5), and

(2) All animals received a second IM injection of 1500 IU of PMS on day 16 following breeding.

The same 2500 IU of HCG was injected IV just prior to breeding on day 21 or sooner if the cow was in heat. Cows were left on treatment until they conceived unless they were prone to developing cystic ovaries, or other cyclic abnormalities, in which case they were removed from test. These modifications allowed for:

(1) observation on the effectiveness of the lower level of PMS in producing multiple offspring;

(2) observation on the effect, if any, of omitting the PMS injection on day 5;

(3) the reduction between breeding dates from approximately 6 weeks to approximately 3 weeks for those cows which did not conceive; and

(4) observation on the effect of PMS injection 16 days following breeding on conception rate or subsequent ovarian response.

No cows aborted multiples and only one returned to heat without showing signs of abortion. It was felt, somewhat prematurely, that the response might not be optimum to this level of PMS. It was decided to change the treatments to a higher level to see if more multiples could be obtained and to a lower level to check its effectiveness in producing multiple births and its effect on conception rate.

<u>Trials 3 and 4</u>

At this point cows were alternately assigned as they became available, to one of two levels of PMS on day 16. These levels were 1250 IU (Trial 3) or 1750 IU (Trial 4). At this time cows were no longer given the 2500 IU of HCG when they were bred but were bred only, on day 21 or whenever they displayed heat, whichever came first.

Cows remained on these treatments until the experiment was terminated.

Hormone Treatments - Summary

The end result of the adjustments to the hormone treatment levels resulted in five different trials. The basis of these trials is summarized in Table 2.

TABLE 2. Hormone treatments

Trial No.	IU PMS on day 5	IU PMS on day 16	IU HCG[1]	IU PMS on day 16 following breeding
1A	1500	2000	2500	0
1B	1000	1500	2500	0
2	0	1500	2500	1500
3	0	1250	0	1250
4	0	1750	0	1750

[1] HCG was injected on day 21 or whenever the cow showed estrus, whichever came first.

Animals and Management

The experimental animals were obtained from the dairy herd at the Dairy Research Unit, Edmonton Research Station, University of Alberta, and consisted of grade and purebred Holsteins. Ages ranged from 15 month-old virgin heifers to cows up to 7 years old. Experimental treatments commenced on September 15, 1969. Cows were put on treatment as they became available following calving. Heifers were put on treatment at approximately 15 months of age or whenever they appeared to be cycling normally. Throughout the hormone treatments cows and heifers were handled as they normally would have been. Milking cows received a concentrate ration in proportion to their production. During the summer months cattle were on pasture;

during the winter they received roughage indoors individually or outdoors as a group, depending on weather conditions.

After 6 weeks without signs of heat, pregnancy was diagnosed by rectal palpation by a veterinarian. No attempt was made to determine the number of follicles developed, number of ovulation points or number of developing embryos. On several occasions pregnancy diagnosis revealed that both horns of the uterus were enlarged and contained developing fetal membranes; however, no attempt was made to determine the number of embryos in each horn. Since most superovulation studies where the animals are not slaughtered involve extensive rectal examinations, it was considered that a minimum number of examinations might improve the chances of the cow carrying her offspring to term.

Experimental animals were weighed on the first Wednesday following breeding, and on the first Wednesday of every month for those diagnosed as pregnant. Commencing twelve weeks before the expected calving date (calculated as 278 days from breeding), the cows were weighed at weekly intervals. Cows were also weighed as close as possible to calving and again after calving, in most cases after passage of the afterbirth. The body condition of each cow was rated at twelve weeks prior to the expected calving date and again at calving. A condition score of 1 to 5 was used with 1 being extremely thin and 5 being extremely fat.

The amount of roughage and concentrate fed was recorded during the final twelve-week period. Each cow was allowed free-choice of roughage in the first week. Thereafter, body weight was reviewed weekly and the level of concentrate adjusted in an attempt to maintain an adequate degree of body condition. The amount of gain expected was modified by the capacity of the cow to eat the necessary amount of feed and by the condition of the cow. From the time the first cow started on feed on May 6, 1970, until November 11, 1970, cows were fed brome-alfalfa hay as roughage. Oat greenfeed was fed from November 11, 1970 until the last cow calved on January 17, 1972. The composition of the concentrate is shown in Table 3. Nutritional comparisons were based on the number of offspring eventually produced and not on the hormone level given.

During the last 12 weeks of gestation cows were fed individually in stalls with metal grates over a liquid manure gutter. Weather permitting, cows were allowed exercise in a small corral where no feed was available. Water and a mixture of cobaltized-iodized salt and dicalcium-phosphate were available free-choice.

Calves were weighed at birth and every Wednesday thereafter until they were 16 weeks old (nearest Wednesday). Whole milk was fed twice daily for five days at 10% of birth weight. Thereafter a milk substitute was fed twice daily to a maximum of 600 grams per day until the end of the fourth week, twice daily at 450 grams per day for the fifth week, and once daily at 200 grams per day for the sixth week. The milk replacer was mixed 1:7 with water. From approximately one week of age, calves had access to a calf meal, the composition of which is shown in Table 3. Calves did not receive additional roughage. Calf weights and feed consumption were recorded.

TABLE 3. Ration formulations and analyses

Ration ingredients (kg or %)	Calf meal	Cow concentrate	Brome-alfalfa hay	Oat greenfeed
Beet pulp		9.0		
Molasses	3.0			
Oats	15.0	13.5		
Wheat	57.0			
Barley		60.0		
Soybean meal	20.0	12.5		
Alfalfa meal	3.0			
Limestone	0.5			
Cobaltized-iodized salt	0.5	0.5		
Tri-sodium-polyphosphate		3.5		
Vitamin mix[1]		1.0		
Vitamin-antibiotic mix[2]	1.0			
	100.0	100.0	100.0	100.0
Analyses-Actual				
Dry matter %	86.78	90.78	92.25	92.04
Crude protein (air dry basis) %	18.90	13.60	13.69	11.89
Gross energy (air dry basis)(Mcal/kg)	4.00	3.99	4.21	4.00

1 Formulated to supply 6600 IU Vit A, 660 IU Vit D, and 66 IU Vit E per kg of ration.

2 Formulated to supply 8800 IU Vit A, 880 IU Vit D, 88 IU Vit E and 33 mg of Aureomycin per kg of ration.

Digestibility Trials

Digestibility trials using four lambs were carried out on samples of all feedstuffs eaten by both cows and calves. The lambs were kept in metabolism cages and fed each feedstuff (hay, greenfeed, cow concentrate, and calf meal) for a three-week adjustment period followed by a one-week metabolism study. Feed intake was recorded for the first 5 days of the metabolism study while fecal output was recorded for the last 5 days. Feces were weighed daily and 20% of the weight was taken as a sample and frozen for later drying and further analysis.

Analytical Methods

Dry matter (DM) and crude protein were determined on the feed and fecal samples by AOAC (1965) methods.

Gross energy of feed and fecal samples was determined by combustion at 25 atmospheres of oxygen in a Parr oxygen bomb calorimeter.

Statistical Analyses

An IBM MTS computer in the Department of Computing Science, University of Alberta was used to statistically analyze the data. A one-way analysis of variance APL program, ANOVA2 (Smillie, 1969), was used to determine F values for means. Significance of F values was determined by methods outlined by Steel and Torrie (1960).

RESULTS AND DISCUSSION

Hormone Treatments

Trial 1A

Trial 1A consisted of an IM injection of 1500 IU of PMS on day 5, an IM injection of 2000 IU of PMS on day 16 and an IV injection of 2500 IU of HCG on day 21 or whenever the cow showed heat, whichever came first.

Twenty of 53 cows treated were diagnosed as pregnant. No cow was treated more than once, so all 20 cows conceived to first service. Conception to first service of 37.7% compares to the previous herd average for January 1, 1960 to September, 1969 of 43.7 percent. Information on those cows which conceived in Trial 1A is shown in Table 4. A more complete explanation of the column headings for Tables 4 to 9 is available in Appendix 1.

In addition to the 53 cows listed as being treated in Trial 1A, two other cows were also treated. These two cows later proved to be pregnant to a service prior to the initiation of treatment. One of these cows, diagnosed to have been about ten weeks pregnant at the time of treatment, aborted a single fetus 34 days after she was bred in Trial 1A. There was no indication that the hormone treatment was directly responsible for the abortion. The other cow gave birth to a single calf at a date which corresponded to her last breeding prior to going on treatment; i.e. six weeks before her treatment breeding.

Only 13 calves were born alive to the 20 cows diagnosed as pregnant, resulting in a 65% calf crop. This included one set of quadruplets which died shortly after birth, two sets of twins and

TABLE 4. Data of cows conceiving in Trial 1A

Cow No.	Breeding interval in days	Services to treatment conception	Calves born or aborted	Calves born alive	New lactation
6541	132	1	1	1	Yes
6630	73	1	1	1	Yes
6345	128	1	1	1	Yes
6803	76	1	1	1	Yes
6720	72	1	1	1	Yes
6752	78	1	2	2	Yes
6615	76	1	2	2	Yes
6518	101	1	5	0	Yes
6718	70	1	6	0	Yes
6839	(0)[1]	1	3	0	Yes
6715	54	1	3	0	Yes
6640	149	1	3	0	Yes
6901	(0)[1]	1	4	4	Yes
6721	163	1	1	0	No[2]
6730	77	1	1	0	No[2]
6606	72	1	1	0	No[2]
6712	66	1	unknown	0	No[2]
6506	83	1	unknown	0	No[2]
6702	72	1	unknown	0	No[2]
6719	67	1	unknown	0	No[2]

[1] The number of days from first service to the service of conception; in this case the heifer conceived to first service.

[2] The cow was lactating at the time of abortion and continued at about the same level of production.

five singles. Because of the death of the quadruplets only nine calves or a 45% calf crop was actually realized compared to the herd average from January, 1960 to the start of the experiment of approximately 89 percent. Of the cows which did not carry to term, four cows (6712, 6506, 6702, 6719) returned to heat without showing any visible indication of abortion. They gave indications that they were not pregnant, by estrus or excessive vaginal discharge, at 113, 89, 142 and 97 days, respectively, following the treatment breeding to which they had been diagnosed as pregnant. Early abortion of one or more fetuses could have gone unnoticed, since the liquid manure system in the Dairy Cattle Research Unit could easily have prevented observation of placental membranes in the early stages of development. Another three (6721, 6730 and 6606) of the seven cows which did not carry to term aborted single fetuses at 94, 105 and 168 days of gestation, respectively.

Three of the 13 cows which calved at term or near term, resulting in a new lactation, produced triplets. Parturition occurred at 246 (Figure 2), 261 and 274 days of gestation. All were dead at birth and had probably been dead in utero for at least 24 hours before birth. One cow produced 5 fetuses at 151 days, all of which appeared normal for their stage of gestation. Laboratory tests showed no pathological or genetic abnormalities. The remaining cow produced 6 normal fetuses at 209 days of gestation. (Figure 3).

The single set of quadruplets was taken by Caesarean at 261 days of gestation (Figure 4). All died about 20 minutes after delivery. The two sets of twins born alive after 280 and 288 days of gestation were normal at birth.

FIGURE 2. TRIPLET CALVES BORN TO COW 6839 AFTER 246 DAYS OF GESTATION

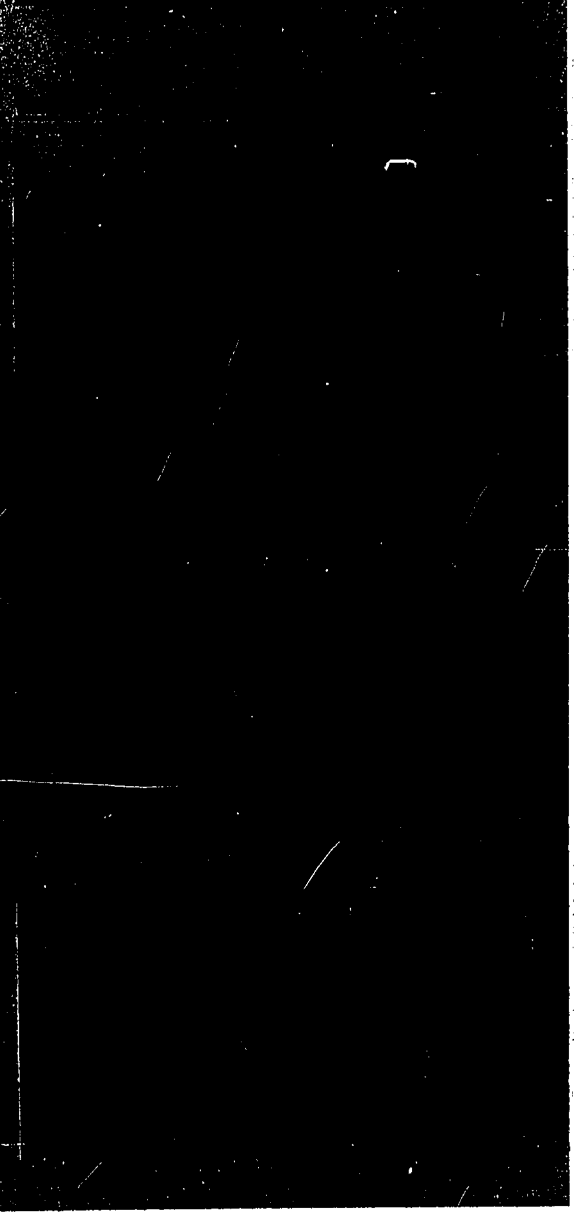

FIGURE 2. TRIPLET CALVES BORN TO COW 6839 AFTER 246 DAYS OF GESTATION

FIGURE 3. SIX FETUSES BORN TO COW 6718 AFTER 209 DAYS OF GESTATION

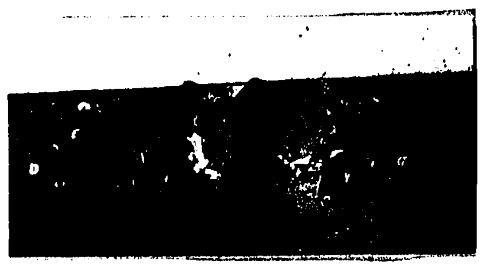

FIGURE 4. FOUR CALVES TAKEN BY CAESAREAN DELIVERY AT 261 DAYS OF GESTATION FROM COW 6901

FIGURE 5. DEFORMED CALF TAKEN BY CAESAREAN DELIVERY AT 218 DAYS OF GESTATION FROM COW 6721 (Only the front half is shown; the back portion was dismembered in attempts to deliver the fetus.)

Of the 13 term or near term births in Trial 1A, 2 were twin births resulting in a twinning percentage of 15.4. Multiple births in excess of twins were six out of thirteen or 46.2 percent. Multiple births accounted for 61.5% of the calvings. Thirty-two of the 33 cows which did not conceive in Trial 1A were placed in Trial 1B as outlined previously.

Trial 1B

Trial 1B consisted of an IM injection of 1000 IU of PMS on day 5, an IM injection of 1500 IU of PMS on day 16 and an IV injection of 2500 IU of HCG on day 21, or sooner if the cow came into heat. All cows receiving Trial 1B had been given Trial 1A and failed to conceive, or, in several instances, had conceived and returned to heat shortly after being diagnosed as pregnant at about 6 weeks.

Nine cows conceived to first breeding in Trial 1B and five cows conceived to second breeding from a total of 48 treatments given to 32 cows. Data of those cows conceiving in Trial 1B are shown in Table 5. Conception to first service was 28.1 percent. Of the 14 cows conceiving in Trial 1B, 11 carried to term or near term resulting in new lactations. Eight single calves, 2 sets of twins and 1 set of triplets were born. The triplet birth included 2 normal term calves which were dead and a single fetus of about 100 days gestation which was in the process of resorption. One of the single births was a Caesarean delivery, after 218 days of gestation, of a deformed male calf with a large growth of muscle tissue on its neck (Figure 5). It was dismembered in attempts at delivery. The calf appeared to have been dead in utero for at least several days.

TABLE 5. Data of cows conceiving in Trial 1B

Cow No.	Breeding interval in days	Services to treatment conception	Calves born or aborted	Calves born alive	New lactation
6721	317	1	1	0	Yes
6712	218	1	2	2	Yes
6735	109	1	1	1	Yes
6614	109	1	1	1	Yes
6537	199	2	1	1	Yes
6745	(261)[1]	2	1	1	Yes
6347	167	2	1	1	Yes
6836	(117)[1]	2	1	1	Yes
6828	(70)[1]	2	1	1	Yes
6802	(233)[1]	1	3	0	Yes
6727	150	1	2	2	Yes
6825	(58)[1]	1	1	0	No[2]
6730	248	1	6[3]	0	No[4]
6806	(265)[1]	1	unknown	0	No[2]

1 The number of days from first service to the service of conception.

2 Heifers did not give sufficient milk at the time of their abortion to start a lactation.

3 Six were found; there could have been more.

4 The cow was lactating at the time of the abortion and continued at about the same level of production.

Twinning percentage on the basis of term or near term births was 18.2 while multiples in excess of twins was 9.1 percent.

Three cows did not go to term. Cow 6825 aborted a single fetus at 193 days and cow 6806 returned to heat after 147 days without showing any signs of abortion. Cow 6730 aborted at least six fetuses at 44 days of gestation. This cow had not been diagnosed as pregnant, nor was she seen to have aborted; however, on September 16th, 1970, when membranes were found in the corral containing six fetuses approximately six weeks of age, this was the only cow which was at the appropriate stage to have aborted fetuses of this size. She returned to heat 33 days after the suspected abortion.

Calving percentage on the basis of cows conceived was 78.6. One calf (single) died within two weeks of birth reducing the effective calf crop to 71.4 percent.

As explained earlier, cows were allowed a maximum of 2 cycles under Trial 1B. Because of cystic ovaries or other cyclic abnormalities, many cows received only 1 treatment under this regime. Fifteen of the 18 cows which did not conceive in Trial 1B were removed temporarily from the experiment because of abnormal estrus cycles.

Trial 2

A total of eight cows conceived in Trial 2 out of 48 treatments given to 29 cows. Five of these were to first service for a conception rate of 17.2% to first service. Nine of the 29 cows had not been given any previous treatment; the remaining 20 had received either Trial 1A or both 1A and 1B. Of the 9 cows which had not received any previous treatment only 2 settled to first service.

Data from those cows conceiving in Trial 2 are presented in Table 6.

TABLE 6. Data of cows conceiving in Trial 2

Cow No.	Breeding interval in days	Services to treatment conception	Calves born or aborted	Calves born alive	New lactation
6537	54	1	1	1	Yes
6516	113	3	1	1	Yes
6541	87	1	1	1	Yes
6506	293	2	1	1	Yes
6719	213	2	1	1	Yes
6350	91	1	1	1	Yes
6614	82	1	2	2	Yes
6702	234	1	unknown	0	No[1]

1 The cow was lactating at the time of abortion and continued at about the same level of production.

Of the 8 cows which conceived, 7 went to term producing 8 calves for a calving percentage of 100. Six cows had singles and one cow had twins, resulting in a twinning percentage of 14.3 on the basis of term births. One cow returned to heat after 149 days without showing signs of abortion.

The 21 cows which did not conceive in Trial 2 were either removed from test or were placed on one of Trials 3 and 4. The low conception rate in Trial 2 and the premature, and apparently incorrect, belief that a slightly higher level might produce a more optimum response led to the initiation of Trial 4. The lower level in Trial 3 was an attempt at evaluating the response to an even lower level of PMS.

Trial 3

A total of 11 cows conceived in Trial 3 out of 37 treatments given to 20 cows. Three of the cows had received no previous treatment, the remaining 17 had received at least one treatment in Trials 1A, 1B or 2. Data from cows conceiving in Trial 3 are shown in Table 7.

Of 11 cows conceiving, 8 conceived to first service in Trial 3 for a conception to first service of 40 percent. Of the 11 cows conceiving 9 carried to term or near term. One cow which did not carry to term returned to heat after 185 days with no sign of abortion. This cow (7702) had conceived in Trial 1A and returned to heat and did the same thing in Trial 2. The second cow aborted a single fetus at 163 days.

Cow 7727 delivered dead twin calves at 202 days of gestation. This cow had given birth to twins in Trial 1B. The 9 cows which went to term produced 9 calves for a calving percentage, on the basis of cows conceiving, of 81.8. Births consisted of 7 singles and 2 sets of twins for a twinning rate of 22.2% on the basis of births resulting in new lactations.

Trial 4

Thirteen cows conceived to 38 treatments given to 19 cows. One of the cows had received no previous treatments; the remaining cows had received at least one treatment in previous trials. Data of cows conceiving in Trial 4 are shown in Table 8.

Of the 13 cows conceiving, 7 conceived to first service for a conception to first service of 36.8 percent. Only 6 of these 13 cows

TABLE 7. Data of cows conceiving in Trial 3.

Cow No.	Breeding interval in days	Services to treatment conception	Calves born or aborted	Calves born alive	New lactation
6451	191	1	1	1	Yes
6931	(50)[1]	1	1	1	Yes
6941	(0)[1]	1	1	1	Yes
6724	133	3	1	1	Yes
6542	80	1	1	1	Yes
6630	70	1	1	1	Yes
6606	309	1	1	1	Yes
6727	138	3	2	0	Yes
6905	(247)[1]	1	2	2	Yes
6802	108	2	1[2]	0	No[3]
6702	403	1	unknown	0	No[3]

1 The number of days from first service to the service of conception.

2 One was found on pasture, there could have been more.

3 The cow was lactating at the time of the abortion and continued at about the same level of production.

carried to term. The seven which did not carry to term included one cow which died of hardware disease at about 6.5 months of gestation, four cows which returned to heat at 125, 143, 74, and 92 days after breeding without showing any signs of aborting, and 2 cows which aborted at 127 and 181 days of gestation. The number of fetuses aborted from these 2 cows is unknown since the cows were on pasture at the time and fetuses could not be found. Five cows gave birth to single calves and one cow gave birth to triplets, only 2 of which were alive at birth. The cow giving birth to triplets had produced

TABLE 8. Data of cows conceiving in Trial 4

Cow No.	Breeding interval in days	Services to treatment conception	Calves born or aborted	Calves born alive	New lactation
6602	175	1	1	1	Yes
6913	(159)[1]	3	1	1	Yes
6920	(163)[1]	2	1	1	Yes
6745	100	1	1	1	Yes
6828	83	2	1	1	Yes
6718	75	1	3	2	Yes
6820[2]	228	1			
6735	129	2	unknown	0	No[3]
6825	(458)[1]	2	unknown	0	No[4]
6730	442	3	unknown	0	No[3]
6943	(0)[1]	1	unknown	0	No[4]
6729	73	1	unknown	0	No[3]
6803	64	1	unknown	0	No[3]

1 The number of days from first service to the service of conception.

2 Died of hardware disease at about 6.5 months of gestation.

3 The cow was lactating at the time of abortion and continued at about the same level of production.

4 The heifer did not produce sufficient milk as a result of the abortion to start a lactation.

6 fetuses in Trial 1A. Percent multiple births was 16.7. Calving percentages on the basis of cows conceiving was 53.8.

Non-treated Cows

A number of cows were treated in Trials 1A and 1B, failed to conceive and were therefore bred as they displayed heat. Most were not placed in Trial 2 because of cyclic irregularities which may have been caused or aggravated by the previous treatments. A few missed their injection on day 16 and were bred when in heat, thus essentially receiving no treatment even though they were supposed to. A few others toward the end of the hormone treatment period came into heat more than ten days after their FSH injection, were bred and were therefore considered not to have received any treatment. The results of cows conceiving in this group are shown in Table 9. One cow died of pneumonia before term but all other 18 cows went to term and all produced single calves. One calf was born dead and another died at about 2 weeks of age. Calving percentage on the basis of cows conceiving, disregarding the cow which died of pneumonia, was 94.2 born alive, or 88.9 alive shortly after birth.

TABLE 9. Data of cows not on treatment at conception

Cow No.	Breeding interval	Services to conception on treatment	Gestation length	Calves born or aborted	Calves born alive	New lactation	Days from treatment calving or abortion to return conception	Services to return conception
643	256	1	161[1]	1	0	No	died	
610	182	1	284	1	1	Yes	83	1
910	(336)[2]	1	292	1	0	Yes	99	3
726	161	1	283	1	1	Yes	229	5
645	177	1	278	1	1	Yes	178	4
603	249	1	284	1	1	Yes	199	5
724	288	1	284	1	1	Yes	135	4
729	234	1	277	1	1	Yes	73	3
542	392	1	285	1	1	Yes	80	1
826	(166)[2]	1	280	1	1	Yes	102	2
646	271	1	287	1	1	Yes	sold	
832	(88)[2]	1	279	1	1	Yes	68	1
809	160	2	282	1	1	Yes	237	6
648	182	1	291	1	1	Yes	81	2
834	(162)[2]	1	277	1	1	Yes	59	1

TABLE 9. Data of cows not on treatment at conception (continued)

Cow No.	Breeding interval	Services to conception on treatment	Gestation length	Calves born or aborted	Calves born alive	New lactation	Days from treatment calving or abortion to return conception	Services to return conception
518	188	1	280	1	1	Yes	sold	
911	(241)[2]	1	280	1	1	Yes	98	1
906	(105)[2]	2	277	1	1	Yes	sold	
902	(127)[2]	1	291	1	1	Yes	108	2

1 The cow aborted before dying of pneumonia.

2 The number of days from first service to the service of conception.

Summary and Discussion of Trial Results

A summary of the data available from the 5 PMS trials is presented in Table 10. The percentage of cows conceiving to first service in the University of Alberta dairy herd has usually been low; it was 43.7% from January 1, 1960 to September, 1969 based on the records available. Pelissier (1972) reported an average percentage conception to first service of 44.2 in a number of dairy herds. Gordon et al. (1962) reported a conception to first service of 76.2% in cows treated with PMS at levels varying between 800 and 2000 IU. He did not find any marked differences in conception percentages between any of the levels studied. While conception to first service in all treatment groups on the present study was lower than that reported by Gordon et al., there was no consistent indication that it was lower than the previous herd average, or that differences between trials were real.

Services per conception were 2.65, 3.43, 6.00, 3.36 and 2.92 for cows treated in Trials 1A, 1B, 2, 3 and 4 respectively. In the study of a number of dairy herds, Pelissier (1972) reported an average of 2.77 services per conception. The services per conception in this experiment are consistent with the low percentages of conception to first service. No single reason could be isolated to explain the particularly low percentage conception to first service and correspondingly high services to conception for those cows in Trial 2.

Heat detection was difficult, with many cows showing only minimal signs of estrus. Although not designed to compare the symptoms of heat in treated and non-treated animals, there was no

TABLE 10. Summary of Trial results

Trial	No. of animals treated	Treat-ment given	Conceived to first service	Total con-ceived	% Conceived to first service	Services for conception on treatment	Breeding interval of mature cows (days)	Cows calving
1A	53	53	20	20	37.7	2.65	89.4	13
1B	32	48	9	14	28.1	3.43	189.6	11
2	29	48	5	8	17.2	6.0	145.9	7
3	20	37	8	11	40.0	3.36	179.0	9
4	19	38	7	13	36.8	2.92	152.1	6
Non-treated	-	-	-	19	-	-	228.3	18
Results at U. of A. 1960-69	-	-	122	310	43.7	-	-	306

TABLE 10. Summary of Trial results (continued)

Trial	Calves born	Calves born alive	Cows abort-ing	Calves aborted	Cows returning to heat	Calv-ing % (alive shortly after birth)	% Twin births	% Multi-ples above twins	Total % multiple births	Ave. breeding interval after treatment calving (days)
1A	33	13	3	3	4	45.0	15.4	46.2	61.6	81.2
1B	15	11	2	7	1	78.6	18.2	9.1	27.3	110.4
2	8	8	0	0	1	100.0	14.3	0.0	14.3	127.5
3	11	9	1	1	1	81.8	22.2	0.0	22.2	66.4
4	8	7	2	unknown	4	53.8	0.0	16.7	16.7	129.3
Non-treated	18	17	1	1	0	94.4	0.0	0.0	0.0	121.1
Results at U. of A. 1960-1969	322	276	-	-	-	89	4.58	0.33	4.90	

obvious evidence that symptoms of heat were suppressed or enhanced in treated animals compared to the symptoms expressed by animals before the experiment started. Pelissier (1972) reported heat detection to be a problem in many herds. Gordon et al. (1962) indicated that incidence of silent heat is greater in cows immediately after treatment with PMS than in non-treated animals and that low levels (800 IU) appear more prone to produce silent heat than higher levels (1600-2000 IU).

For several years prior to the start of the experiment silent heat, cystic ovaries and other reproductive abnormalities had been prevalent in the University dairy herd. This was attributed largely to the fact that the herd size was being doubled through natural herd replacements and culling was minimal. For this reason it is difficult to determine if the hormone treatments produced a greater degree of cyclic abnormalities and lower conception rates than was already present.

The shortest breeding interval, 89.4 days for cows in Trial 1A, was expected since cows were allowed only one service in this trial. The longest breeding interval, 228.3 days for cows in the non-treated group, was also expected since this group consisted primarily of problem cows which had failed to conceive in one or more of the PMS treatment groups. The breeding interval of mature cows in Trials 1B, 2 and 3 did not appear to be different.

Calving percentages, when calculated on the bases of cows conceiving, were 45.0, 78.6, 100.0, 81.8 and 53.8% in Trials 1A, 1B, 2, 3, and 4, respectively, compared to 94.4% for the cows conceiving while not on treatment and to the approximate figure of 89% for the

University dairy herd from January 1, 1960 to the start of the experiment. Trials 1A and 4 received the highest levels of PMS on day 16, 2000 IU and 1750 IU, respectively, but these two treatments yielded the lowest calving percentages. The calving percentages in Trials 1B, 2 and 3 are not sufficiently high to consider these hormone treatments as methods of increasing calf crop percentages. The groups receiving the high levels of PMS produced no more twin births than the lower levels but did result in a greater proportion of multiple births above twins, the majority of which were dead, than groups receiving the lower levels of PMS. These results would agree with those of Gordon et al. (1962) who found that higher levels of PMS produced extra twin ovulations but a higher proportion of cows with more than 2 eggs shed.

Gordon et al. (1962) reported, in addition to finding a greater response at higher levels of PMS (1600 and 2000 IU), that the percentage of cows suffering total fetal loss was higher where higher numbers of ova were released. His results ranged from a 16.4% loss in cows shedding two eggs to a 100% loss in cows shedding 17-25 eggs. Similarly, in the present study cows given the higher levels of PMS had the greatest number of returns to estrus following pregnancy diagnosis. The average number of days to return estrus from the date of conception for these cows was 123.3 days. Gordon et al. (1962) found that the majority of cows returning to estrus did so in less than 120 days from conception.

The injection of PMS on day 5 of the cow's cycle (Turman et al., 1969) was based on results obtained by Schilling and Holm (1963) who felt that a number of follicles begin to develop shortly after estrus but that all but one became atretic. The low level of PMS early in the cycle was designed to prevent the atrecia of follicles. There was no indication in the present study that an injection of PMS on day 5 was beneficial in improving either conception rates or calf crop percentages. Turman et al. (1971) also found no obvious benefit from the injection of PMS early in the cycle.

Some researchers have injected HCG at breeding (Hafez et al., 1963; Scanlon et al., 1968) to ensure ovulation of all follicles, although it is generally felt that endogenous LH in the cow is adequate for ovulation of any number of mature follicles which might develop (Gordon et al., 1962). The injection of 2000 IU of HCG just prior to breeding of cows in Trials 1A, 1B and 2 did not appear to give any improvement in conception or in calf crop percentages when compared to cows in Trials 3 and 4 which did not receive this injection. More recently, Laster et al. (1971) has reported that an injection of HCG three days after the PMS injection on day 16 produced a greater number of ovulations of two ova than ovulations of three ova when compared to the response obtained when the HCG injection was given at the time of estrus and breeding. Improvements in technique such as this may lead to improved results over those obtained in this experiment.

Roussel and Beatty (1970) recommended the injection of 500 IU of PMS on day 15 or 16 following breeding to improve conception rate. The injection of PMS in Trials 2, 3 and 4 in this experiment would take advantage of any beneficial effect on conception rate, and, if the cow did not conceive, superovulation for the next estrus would result. Data of cows receiving the injection of PMS on day 16 after breeding did not appear to change conception rate when compared to those animals in Trials 1A and 1B which did not receive this injection. The interval between services was reduced from 6 to 3 weeks for those cows which did not conceive to the first service.

The hormone treatments gave such variable results that these treatments can only be regarded as experimental. The only encouraging trends observed were that the injection on day 5 seemed unnecessary and that the injection on day 16 following breeding was beneficial in shortening the time between services. Both of these can be regarded only as trends in simplifying the treatment.

Refractoriness to injections of PMS may have occurred but it did not appear to be a problem. Cow 6718 produced 6 fetuses following a single injection of 2000 IU of PMS; following an IM injection of 1750 IU of PMS her next conception produced triplets. Cow 6905 received 4 consecutive treatments of 2000, 1500, 1500 and 1500 IU of PMS. Following her fifth treatment of 1250 IU of PMS she conceived and subsequently produced twins. Cow 6614 produced a single calf after receiving two treatments; the first an IM injection of 2000 IU of PMS and the second of 1500 IU of PMS. Following her next conception, after a single treatment of 1500 IU of PMS, she produced twins. Cole _et al_. (1957) and Gordon _et al_. (1962) both found that refractoriness was not generally a problem.

There was no indication of a relationship between weight or age and response to PMS treatment. Of cows conceiving in Trial 1A, for example, #6345, a very large cow, and #6720, a small cow, both produced single calves after PMS treatment. Similarly, #6718, a large cow, produced 6 fetuses, while #6839 and #6901, both small cows, produced 3 and 4 fetuses respectively. The year of birth of these cows is reflected in the first two digits of their identification number. Using this as an indication of age, no correlation between age and response is evident in the cows used in this experiment. Gordon et al. (1962) found that the age of the cow was not of major importance in determining response but that small animals tended to display a greater response than large animals.

Calving Results

Data of cows which produced multiple offspring at or near term and commenced a new lactation are presented in Table 11. Similar data of cows producing single calves after treatment are presented in Table 12, and of cows producing singles in the absence of any hormone treatment in Table 9. The average gestation length for single-born calves was 281.4 days. The twins born dead after a gestation period of 202 days were not included in calculations of the average gestation length for twins. Average gestation length for the remaining six twin pregnancies was 278.0 days. The average gestation length for triplets was 264.8 days. Hewitt(1934) reported an average gestation length in Friesians of 282.7 days for singles and 272.7 days for twins. Gordon et al. (1962) reported an average gestation period for twins and triplets of 273.2 and 262.1 days respectively.

More assistance was given to multiparous cows at calving than to cows producing singles, although in some cases more assistance was given than was necessary. Of the cows producing twins, only cow 6752 required considerable assistance. The first calf was born backwards,

TABLE 11. Data of cows producing multiple offspring

Cow no.	Gestation length	Calves born	Calves born alive	Days to return conception	Services to return conception
6901	261	4	4	died	
6752	280	2	2	sold	
6615	288	2	2	died	
6712	277	2	2	98	3
6802	283	3	0	108	3
6727	268	2	2	138	3
6614	276	2	2	sold	
6518	151	5	0	188	5
6718	209	6	0	75	1
6839	246	3	0	sold	
6715	261	3	0	145	3
6640	274	3	0	sold	
6727	202	2	0		5[1]
6905	279	2	2	74	2
6718	260	3	2	188	6

[1] Received 5 services up to the time of writing but had not yet conceived.

accounting for most of the extra assistance required. Cow 6614 required no assistance. Assistance given to other twinning cows was minimal. A veterinarian was called for most of the multiple births in excess of twins. Since most were somewhat premature it was anticipated, when the cows showed signs of calving, that more than one calf was present.

TABLE 12. Data of cows producing singles on treatment

Cow No.	Gestation length	Calves born	Calves born alive	Days to return conception	Services to return conception
6541	284	1	1	87	1
6630	283	1	1	70	1
6345	287	1	1	56	1
6803	277	1	1	64	1
6720	281	1	1	77	1
6721	218	1	0	sold	
6735	274	1	1	129	2
6614	276	1	1	82	1
6537	280	1	1	54	1
6745	282	1	1	100	1
6347	295	1	1	sold	
6836	283	1	1	75	2
6828	281	1	1	83	2
6537	278	1	1	sold	
6516	282	1	1	sold	
6541	285	1	1	164	5
6506	278	1	1	132	2
6719	276	1	1	214	1
6350	282	1	1	sold	
6451	289	1	1	sold	
6931	280	1	1	50	1
6941	275	1	1	89	2
6724	288	1	1	89	2
6542	282	1	1	died	

TABLE 12. Data of cows producing singles on treatment (continued)

Cow no.	Gestation length	Calves born	Calves born alive	Days to return conception	Services to return conception
6630	276	1	1	sold	
6606	274	1	1	30	1
6602	281	1	1	sold	
6913	276	1	1	181	5
6920	272	1	1	98	3
6745	282	1	1	62	1
6828	277	1	1	117	3

The quadruplets were taken by Caesarean from cow 6901 before she showed any signs of calving; this cow had stopped eating and had serious difficulty getting up and lying down because of an extremely large girth. External massage kept the calves alive for about 20 minutes. The cow died a few days later from metritis and peritonitis resulting from the Caesarean section. Cow 6615 was killed because of gangrenous mastitis about one month after producing twins. Retained afterbirth did not appear to be a problem in cows producing multiple offspring when compared to cows producing single calves.

Assistance given to cows producing single calves was due primarily to difficult deliveries caused by large calves. Cow 6721 produced a deformed male calf (Figure 2) which was taken by Caesarean. It is not known if the hormone treatment was responsible in any way for the deformity. This cow was later sold because uterine adhesions from the Caesarean prevented further conceptions.

Of 15 cows producing multiples, 9 animals or 60.0% remained in the herd, 2 animals or 13.3% died as a result of the birth, 1 cow or 6.7% was killed because of foot rot and arthritis, 2 cows or 13.3% were sold for low production and failure to conceive by 3 services, and 1 cow or 6.7% was sold because of low production alone. For those cows remaining in the herd after producing multiples, the average number of days to return conception and the average number of services per conception was 126.8 and 3.25, respectively. The percentage of animals remaining in the herd following a multiple birth was somewhat higher than the 38.46% reported by Pfau et al. (1948).

Thirty-nine cows out of 51 cows producing single calves or 76.5% remained in the herd after the single birth. Three cows or 5.9% were sold or died because of the parturition, two cows or 3.9% were sold for other health reasons, two cows or 3.9% were sold because of low production and poor reproductive performance, four cows or 7.8% were sold because of low production, and 1 cow or 2.0% was sold because of old age. Figures for days from calving to conception and services per conception for those remaining in the herd were 105.4 and 2.21, respectively. Neither days to return conception nor services to conception were different ($P < 0.05$) between cows producing multiples and cows producing singles.

Digestibility Trials

Data from digestibility trials on the feedstuffs used in this study are presented in Table 13.

The coefficients of apparent digestibility of the calf meal used in this experiment are slightly above the corresponding digestibility coefficients of the calf meals used by Ndyanabo(1971)

TABLE 13. Coefficients of apparent digestibility

	Brome-alfalfa hay	Oat greenfeed	Cow concentrate	Calf meal
Dry matter digestibility (%)	59.82	62.49	79.25	80.90
Crude protein digestibility (%)	57.49	67.71	69.81	77.63
Gross energy digestibility (%)	56.88	62.36	77.52	80.90

and very close to the results obtained by Milligan and Grieve (1970). Malmberg (1972) reported apparent digestiblities for dry matter, crude protein and gross energy of 81, 75 and 80%, respectively, in a concentrate ration fed to growing Holstein calves.

Hoogendoorn (1968) and Wiktorsson (1971) both reported that changes in roughage:concentrate ratio did not change the coefficients of apparent digestibility significantly. The coefficients of apparent digestibility of dry matter and gross energy were very similar in the brome-alfalfa hay and the oat greenfeed used in this study. The coefficient of apparent digestibility of crude protein was slightly higher in the greenfeed than in the hay; however, the amount of each roughage consumed by cows producing multiple offspring and those producing singles was very similar so they are expressed together as 'roughage' (Table 14).

The coefficient of apparent digestibility of energy in the brome-alfalfa hay was slightly lower than data reported by Grieve and Winchell (1972), whereas comparable data for oat greenfeed agreed very closely. The coefficient of apparent digestibility of energy in the cow concentrate agreed closely with data for comparable rations reported by Malmberg (1972).

Feed and Energy Intake by Cows

Data of feed consumption and weight changes by cows during the last part of pregnancy are presented in Table 14. Only those cows which were on feed for 70 days or more were included. Cows which were not on feed for at least 70 days were those that calved or aborted at least 3 weeks early. The weight changes of such cows, it was felt, were not representative of weight changes in gestation periods of normal duration.

The average daily consumption of dry matter from roughage and from concentrate was similar in cows producing singles and in cows producing multiples. As a consequence of similar intake by the two groups and a similar proportion of roughage and concentrate, the daily digestible energy (DE) intake per unit of metabolic weight (MW) was not significantly different ($P < 0.05$) between cows producing multiple offspring and cows producing single offspring.

The average weight during the last 3 months of gestation of cows producing singles was 649 kg and of cows producing multiples was 679 kg. Based on NRC (1966) recommendations cows of these weights should receive 33.5 Mcal and 34.6 Mcal of DE daily, respectively, during the last 2-3 months of pregnancy. When converted to a basis of metabolic weight these become 0.26 Mcal of DE per day per unit of metabolic weight. Based on NRC (1971) recommendations, cows of these weights should receive 26.4 Mcal and 27.2 Mcal of DE daily, respectively, during the last 2-3 months of pregnancy. When converted to the basis of metabolic weight, these

TABLE 14. Cow data for last 12 weeks of gestation[1]

	Ave. DM intake from roughage (kg)	Ave. DM intake from concentrate (kg)	Ave. total DM intake /day (kg)	Ave. DE/ unit of MW/day[2] (Mcal)	ADG on feed (kg)	Body condition score	
						at 6 months of gestation	at calving
Dams of singles	7.7	3.6	11.3^{A}	0.26^{A}	1.05^{A}	3.0	3.4^{A}
Dams of multiples	7.8	3.5	11.3^{A}	0.25^{A}	0.87^{A}	3.0	2.7^{B}

1 Only those cows which were on feed for at least 70 days are included. Parturitions occurring before the cow was on feed for 70 days were considered not to be representative of a near normal gestation length.

2 Metabolic weight (MW) is calculated as $\text{weight}_{kg}^{3/4}$

A, B pairs with a common superscript are not significantly different ($P < 0.01$)

become 0.21 Mcal of DE per day per unit of metabolic weight.

Cows were fed the roughage free-choice and were given concentrate to try to maintain body condition and an average weekly gain of about 10 kg. In some instances neither maintenance of body condition nor achievement of the desired weight gain was realized because of the cows inability or reluctance to consume the amount of feed offered. This fact is reflected in the condition score (Table 14) of cows producing multiples at the end of the gestation period. Although these cows consumed as much as the cows producing singles, they did not maintain the same body condition as those producing singles ($P < 0.01$) because they would not consume additional feed. Cows producing multiples at or near term lost an average of 92.0 kg at calving and weighed 19.2 kg less than at the start of the 3-month test period. Cows producing singles lost an average of 62.8 kg at calving and weighed 32.4 kg more than at the start of the 3-month test period.

In spite of the lower body condition score at the end of gestation, and post-calving weights below weights at the start of the 3-month test period, only one cow in the group of cows producing multiples had a body condition score which might be regarded as poor. The digestible energy intake per unit of metabolic weight of cows producing the multiples at or near term in this experiment was considered adequate for the production of healthy calves and for normal subsequent performance of the cow. However, it would not be considered desirable for high producing dairy cows to commence a lactation when carrying very much less body condition than those producing multiples in this experiment.

Therefore, the level of 0.25 Mcal of DE per unit of metabolic weight appears to be close to the minimum requirement. The figure compares closely with the NRC (1966) recommendation of 0.26 Mcal of DE per unit of metabolic weight for pregnant cows.

While the level of DE received by cows producing singles is slightly higher than that recommended by NRC (1971), the increase in body weight by these cows would suggest that satisfactory performance for cows producing single calves could be obtained with the levels recommended by NRC (1971).

Calf Performance

Average birth weights for live single calves and for live multiple calves were 40.4 and 32.2 kg, respectively (Table 15). These differences were significant ($P < 0.01$) and are in close agreement with most research. The average birth weight of multiples was 79.7% of the weight of singles. Turman et al. (1969) reported twin birth weights which were 75.7% of the birth weight of singles; Gordon et al. (1962) reported twin birth weights which were 87.2% of the birth weight of singles. The multiples included in the present study were six sets of twins and 2 calves living from a set of triplets. Average birth weight for the twins alone was 33.5 kg.

The average consumption of whole milk was much higher ($P < 0.01$) for multiple calves than for singles (Table 15). This was due primarily to one set of twins which had an extremely poor performance throughout the 16-week test period and had to be fed whole milk for a total of 37.5 days each, compared to 5 days for most of the other calves. Calves in another set of twins required whole milk for 23 and 8 days before they could safely be weaned, and the surviving triplets received whole milk for 16 days each. Six of the 42 single calves required whole milk for longer than the usual 5 days; however, the longest period was 21 days for one calf. The set of twins which required whole milk for 37.5 days each, and all singles requiring extra whole milk, were on trial at about the same time and suffered from an outbreak of calf scours. The set of twins did not recover sufficiently and were destroyed about a month after the 16-week test period was over.

There was no difference ($P<0.05$) in the amount of milk replacer consumed by singles and by multiples (Table 15) primarily because calves requiring supplemental milk were fed whole milk rather than milk replacer.

Calf meal consumption by single calves was significantly higher ($P<0.01$) than the consumption by calves from multiple births (Table 15). Calves in the multiple birth group required 2.44 kg of calf meal per kg of gain, whch was lower ($P<0.01$) than the 2.65 kg per kg of gain required by single calves. The apparent improved feed efficiency of multiples as compared to singles can be attributed largely to the extra milk received by multiples so that a higher proportion of gains made by this group, particularly in the first several weeks, was made from the milk rather than from the calf meal. Most authors report an improved feed efficiency with increased consumption. Moon (1972), in work with older Holstein steers (average weight was 319 kg at the start of the trial) reported a somewhat improved feed efficiency in those animals with the highest dry matter intake, although differences

TABLE 15. Calf performance

	Ave. birth wt. (kg)	ADG (kg)	Weight at 16 weeks (kg)	Ave. whole milk con-sumption (kg)	Ave. milk replacer con-sumption (kg)	Ave. calf meal con-sumption (kg)	Ave. DE[1] intake/day (Mcal)	Ave. feed efficiency[2]
Singles	40.4^{A}	0.81^{A}	131.0^{A}	22.68^{A}	13.79^{A}	239.1^{A}	7.93^{A}	2.65^{A}
Multiples	32.2^{B}	0.74^{A}	114.5^{B}	34.98^{B}	13.93^{A}	198.8^{B}	6.64^{B}	2.44^{B}

1 DE from calf meal only.

2 Calculated for calf meal consumption only.

A, B pairs with a common superscript are not significantly different ($P < 0.01$).

were not significant. Malmberg (1972), using Holstein steers averaging 170 kg at the start of the trial, found an improved feed efficiency with higher energy levels. Milligan (1967) reported feed efficiencies in Holstein calves from 0 to 120 days of 2.87 to 3.29 kg feed per kg of gain.

The average daily digestible energy intake (Table 15) was higher ($P < 0.01$) for singles than for multiples corresponding to a greater calf meal consumption by single calves than by multiples.

The higher ($P < 0.01$) 16-week weights for singles as compared to multiples was largely due to the higher birth weights of singles, and partly due to the extremely poor performance of one set of twins. If the 16-week weight of this set of twins is excluded from the data, average 16-week weights increase from 114.5 kg to 121.1 kg. The average daily gain (ADG) of 0.81 kg for singles and 0.74 kg for multiples was not significantly different ($P < 0.05$). The ADG reported by Milligan (1967) for Holstein calves ranged from 0.54 to 0.71 kg.

Based on the results shown in Table 15, calves in the multiple birth group were estimated to require an additional 40.3 kg of calf meal and an additional 22.3 days to reach the 16-week weight of singles. Based on anticipated increased consumption, lower feed efficiency and improved ADG with increasing age, the calves in the multiple birth group would probably require slightly more feed and fewer days than was estimated above.

The weight of calf produced per cow for those producing live multiples in the present study was 229.0 kg at 16 weeks compared to 131.0 kg for cows producing singles. The results of this study would indicate that the performance of calves born alive as multiples, while individually slightly below that of singles, is superior on a 'production per cow' basis. This would support the view of Gordon *et al.* (1962) that "twins in cattle, under certain conditions of animal husbandry, is a desirable goal at which to direct further research efforts".

SUMMARY

All hormone treatments used in attempts to obtain a high proportion of twin births were rated as unsatisfactory. A higher degree of superovulation resulted when cows were injected with 1750 and 2000 IU of PMS on day 16 of the estrus cycle than when cows were injected with 1500 or 1250 IU of PMS. However, a high proportion of the cows injected with the 2 higher levels of PMS aborted more than 2 fetuses prematurely or returned to estrus after being diagnosed as pregnant.

No differences in conception rates or in multiple births were noted between groups of cows which received a supplementary injection of PMS on day 5 of the cycle and those cows which did not receive the supplementary injection. Similarly, no differences in conception rates and in multiple births were noted between those groups of cows which received an IV injection of HCG at breeding and those groups of cows which did not receive this injection. Conception rates and numbers of multiple births in those groups which received a superovulating dose of PMS on day 16 following breeding were similar to those in the groups which did not. The injection on day 16 following breeding appeared to offer potential in shortening the interval between services from 6 to 3 weeks for those cows which did not conceive to the first service.

Mortality was high in all multiple birth groups in excess of twins. One set of twins was born dead after a gestation length of 202 days. The remaining twins all lived. Some extra assistance at calving was given to cows with multiple offspring. Although

days to return conception and services per conception were greater for cows with multiple births than for cows with single births, the differences were not significant ($P<0.05$). The number of cows remaining in the herd was 76.5 and 60.0% for those producing singles and those producing multiples, respectively.

Digestibility coefficients were similar to those in other research reports. Digestibility coefficients for the hay and greenfeed were sufficiently similar that they did not have to be evaluated separately in determining digestible energy intake.

The amount of digestible energy received by cows producing single calves agreed more closely with NRC (1966) recommendations than with NRC (1971) recommendations; however, the amount of weight gained by these cows and the increase in their body condition would indicate that cows producing single calves could perform satisfactorily with the lower NRC (1971) recommendation of 0.21 Mcal daily per unit of metabolic weight. Cows producing multiple offspring performed satisfactorily on the amount of digestible energy they received, which was 0.25 Mcal per day per unit of metabolic weight. The corresponding figure recommended by NRC (1971) for single births was 0.21 Mcal per day per unit of metabolic weight, which is probably too low for satisfactory performance by cows producing twins.

The calves born as multiples in this study had significantly lower ($P<0.01$) birth weights, higher ($P<0.01$) whole milk consumption and lower ($P<0.01$) calf meal consumption than calves born as singles. The apparent superior feed efficiency of multiples over singles ($P<0.01$) was due primarily to the higher milk consumption of multiples. Average daily gain for multiples was lower than for singles, however differences were not significant ($P<0.05$). The total production per cow was greater for cows producing multiples than for cows producing singles.

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APPENDIX I Explanation of column headings used in Tables 4 to 9, 11 and 12

Breeding Interval: For mature cows this figure represents the number of days from a cow's previous calving to the service at which the cow conceived. Parturition dates are counted as a calving date if the parturition resulted in a new lactation. If the parturition did not result in a new lactation, the date of the calving prior to that, which did result in a new lactation is taken as the start of the period from calving to conception. For heifers being bred for the first time this figure represents the number of days from first service until the service of conception. For ease in differentiating between mature cows and heifers the figure for heifers is placed in parentheses.

A few cows, injected and bred on day 21, had not shown signs of heat but did show signs of heat a few days later. If this was the case, these cows were rebred when they showed heat. In calculating days between sevices, gestation lengths and number of services of such cases, the service given on day 21 was disregarded if it appeared that the second was a true estrus period. If the cow showed signs of estrus more than 10 days after her day 21 breeding such a heat period was regarded as day 0 and the cow went through a new treatment schedule.

Number of services to conception: The figure given represents the number of services to conception on the treatment being discussed; a number of previous services, on or off treatment, may have been given.

Calves born or aborted: This figure represents the total number of calves or recognizable fetuses dilivered by the cow.

Calves born alive: All calves which showed signs of life (breathing) were designated as being born alive even if they died within a few minutes.

New lactation: If the cow had sufficient udder development and if she appeared to be giving milk a new lactation was started and this is designated as "Yes" in the tables. "No" indicates she did not develop sufficient udder to start a lactation or she was already milking and continued to milk at about the same level.

Days from treatment calving or abortion to return conception: Self-explanatory for most cases. If the cow aborted (did not start a new lactation) and the date of the abortion was known then this figure represents days from the abortion date to the return conception date. If the cow returned to heat; i.e., the abortion date was not known, then tis figure represents the number of days from the last calving or abortion where the date was known, or for heifers the date of the first service. Where abortion dates are not known the figure in this column is followed by an asterisk and for heifers it is placed in parentheses.

APPENDIX II Statistical data

Variable	Source of Variation	Degrees of freedom	Mean squares
Daily DE / unit of met. wt. for cows	Cows with singles vs cows with multiples	1	0.00063
	Error	55	0.00082
	Total	56	0.02864
Ave. total DM/day for cows	Cows with singles vs cows with multiples	1	0.00211
	Error	55	2.3996
	Total	56	1.54905
Condition score at calving	Cows with singles vs cows with multiples	1	3.7556**
	Error	43	0.47804
	Total	44	0.69140
ADG of cows	Cows with singles vs cows with multiples	1	0.24141
	Error	55	0.10412
	Total	56	0.32267
Days to return conception	Cows with singles vs cows with multiples	1	3030.248
	Error	45	2534.905
	Total	46	5034.784
Services to return conception	Cows with singles vs cows with multiples	1	7.2474
	Error	45	2.1746
	Total	46	1.4747
Birth weight	Singles vs multiples	1	704.3809**
	Error	54	24.90697
	Total	55	4.99069
ADG of calves to 16 weeks	Singles vs multiples	1	0.05180
	Error	54	0.019040
	Total	55	0.13799

Variable	Source of Variation	Degrees of freedom	Mean squares
Ave. calf meal consumption	Singles		
	Multiples	1	17048.915**
	Error	54	1439.067
	Total	55	37.9350
Feed efficiency	Singles		
	Multiples	1	0.471276**
	Error	54	0.04888
	Total	55	0.22109
Whole milk consumption	Singles		
	Multiples	1	1586.8542**
	Error	54	325.835596
	Total	55	18.050917
Milk replacer consumption	Singles		
	Multiples	1	0.181569
	Error	54	4.219352
	Total	55	2.054106
16-week weights	Singles		
	Multiples	1	2866.88095**
	Error	54	319.19400
	Total	55	17.86600
DE/day for calves	Singles		
	Multiples	1	17.55635**
	Error	54	1.45539
	Total	55	1.206395

** Significant at the 1% level.