EFFECTS OF HUMAN CHORIONIC GONADOTROPIN ADMINISTRATION AT VARIOUS TIMES FOLLOWING BREEDING ON CORPUS LUTEUM NUMBER, DIAMETER, PROGESTERONE PROFILES AND PREGNANCY RATES IN DAIRY CATTLE.

by

PHARAOH COLLINS SIANANGAMA

B.Sc. Agr. (Hons.), University of Alexandria, Egypt, 1986.

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Department of ANIMAL SCIENCE

The University of British Columbia
Vancouver, Canada

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ABSTRACT

Corpus luteum (CL) dysfunction has been implicated among various factors predisposing early embryonic mortality in cattle. Two experiments were conducted to evaluate the efficacy of using human chorionic gonadotropin (hCG) given either at the time of breeding (day 0), day 7 or 14 post breeding, in reducing that component of early embryonic mortality caused by CL dysfunction. The aims of experiment 1 were to investigate the effectiveness of using hCG, in inducing the development of accessory CL, their formation and growth, and the effect of such treatments on the function of both the induced and spontaneous CL.

Thirty-four lactating Holstein cows were randomly assigned to one of four treatments. A single intramuscular injection of 1000 IU of hCG was given either at the time of breeding (day 0, n=8), day 7 (n=9) or 14 (n=9) post breeding or no hCG given (control, n=8). A real-time ultrasound machine was used to study follicular dynamics and CL growth. The CL and antral follicle diameter was determined using a built-in system of calibrated callipers. Ultrasound scanning was carried out on days 7, 9, 11, 14, 16, 18, 21, 28, 35 and 42 post breeding. Blood and milk samples, for progesterone (P_4) determination using radioimmunoassay, were collected on days coincident with ultrasonography. Diameter of the CL is presented as the sum of the diameter of all luteal tissue in each animal. Differences in CL diameter, milk and plasma P_4 were analyzed using the General Linear Models Procedures while pregnancy data were analyzed using Chi-Square analysis in Statistical Analysis Systems (SAS, version 6.3).

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Based on the day 7 ultrasound scanning, the incidence of twin ovulations was higher among cows treated on day 0 (3/8) compared to control cows (1/8) and day 7 (1/9). Accessory CL were detected in 7/9 of the day 7-treated cows compared to 4/9 among the day-14 treated cows. Least squares means (LSMeans) for CL diameter were significantly higher ($P<0.001$) among cows treated with hCG compared to control cows starting at day 7 continually until day 42. Plasma P_4 profiles were significantly higher ($P<0.05$), at days 18, 35 and 42, in cows treated on day 7 or 14 compared to control cows. The first detectable differences ($P<0.05$) between hCG treated and control cows, in milk P_4 occurred at day 21 and persisted until day 42. Pregnancy rates were highest among cows treated with hCG on day 7 where 6 of the 9 cows were diagnosed pregnant. Corresponding pregnancy rates for day 0, 14 or control cows, were 4/8, 5/9 and 3/8, respectively.

In the second experiment, two trials were conducted at two different farms to investigate the efficacy of using hCG to increase milk P_4 and pregnancy rates. In trial one, 79 lactating Holstein cows were exposed to the treatment protocol described in experiment 1. In addition to the milk sample collection schedule given in experiment 1, a sample was collected on day 0. Milk samples were stored at 4^0C and later transported to the UBC laboratories for P_4 analysis. LSMeans for milk P_4 concentrations were different only at days 16 and 18 post breeding. Pregnancy rates were improved ($P<0.01$) by hCG treatments. The respective pregnancy rates for cows receiving hCG on day 0 (n=20), 7 (n=20), 14 (n=20) or control (n=19) were 25, 35, 35 and 21 %.

In the second trial, 121 lactating Holstein cows were randomly assigned to treatments as described earlier. Weekly milk samples were collected from each animal and assayed for P_4 as described above. LSMeans for milk P_4 were significantly different ($P < 0.05$) among groups starting at day 14 until day 42 post breeding. hCG increased pregnancy rates over control cows. The pregnancy rates for cows treated on day 0, 7, 14 and control were 31, 50, 41 and 26 %, respectively.

In conclusion, this study revealed that treatment with hCG induced accessory CL development, increased P_4 production and improved pregnancy rates. It is evident, too, that treatment with hCG on day 7 post breeding may have greater potential for improving pregnancy rates not only in dairy and beef cattle but equally beneficial to the embryo transfer programmes. Increased pregnancy rates confirm the hypothesis that CL dysfunction does cost the livestock industry appreciable losses in embryos.

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ACKNOWLEDGEMENTS

This thesis is dedicated to

the late **MUNYAMA S. SYANANGAMA**

(R.I.P.)

You are greatly missed.

1. INTRODUCTION

In mammals, the establishment and maintenance of early pregnancy depends on progesterone (P4) which is secreted by the corpus luteum (CL). This support must continue beyond the normal duration of an oestrous cycle. In cattle, results obtained with either natural mating or artificial insemination (AI) fall short of the expected pregnancy rates. Hammond, (1921) suggested that the disparity between the expected and observed pregnancy rates may be due to the demise of embryos or fetuses prior to parturition. Several workers have attempted to elucidate the causative factors predisposing losses in reproductive efficiency. Heap, (1985) wrote "...we are far from understanding which one of these factors is the main causative agent predisposing the demise of embryos early in gestation..."

A considerable number of animals failing to maintain their pregnancies tend to have low P4 concentrations (Bulman and Lamming, 1978). It has been suggested that in these cases, the CL may not be functioning at an optimal level. Hence considerable attention has focused on the effects of an inadequate CL function (Randel et al., 1971; Erb et al., 1976; Bulman and Lamming, 1978). Equally, research efforts have gone into finding solutions to overcome such factors as an inadequate CL function (Hansel et al., 1976; Wagner et al., 1973; McDermott et al., 1986). Consequently, a number of options to resolve this problem have been proposed. Unfortunately, results obtained with any of the various treatment regimen developed thus far, have not conclusively demonstrated that such measures are successful in improving reproductive efficiency in cattle.

The purpose of this study was to attempt to reduce that component of embryonic mortality that may be attributed to an inadequate CL function. To achieve this, it was postulated that supplementing endogenous P4 production can be achieved much more efficiently by inducing the development of accessory CL. It was desired that suitable follicles be present during the times we proposed to administer the treatments. Taylor and Rajamahendran (1990) reported that in the Holstein, as in other breeds of cattle, waves of follicular growth that are a common feature in cyclic cows, are also present in early pregnancy. At the level of ultrasound scanning, no differences could be found between the dominant follicle of the luteal phase and that present during the follicular phase. The dominant follicle of the luteal phase possess receptors to gonadotropins and have been shown to bind the same (Ireland and Roche, 1983). It was hypothesised that the dominant follicle present during early pregnancy is capable of being induced to ovulate.

Under normal physiological conditions, such ovulations are brought about by a surge of the gonadotropin luteinizing hormone (LH). It was, therefore, presumed that if animals were challenged with an ovulatory dose of Human Chorionic Gonadotropin (hCG), the dominant follicle would ovulate. Giving the same dose at the time of breeding was taken to be an assurance that the dominant follicle present at oestrus would ovulate. Being a luteotropin, it seemed reasonable to expect that giving hCG would augment the function of the spontaneous CL.

2. LITERATURE REVIEW

In cattle, acceptable levels of production dictate that cows in their reproductive life conceive every 12-13 months. This demands that cows be rebred and establish pregnancy by 60 days post partum (Nakao et al., 1983). A variety of hurdles, however, stand in between the efforts of farmers and the realization of these goals. First among the obstacles to successful reproduction is the failure of some follicles to ovulate. The incidence of this problem is not known. It has been estimated that fertilization rates in the cow range from 88-90 % (Linares, 1982; Sreenan and Diskin, 1985).

2.1. Extent And Timing Of Embryonic Mortality

Pregnancy rates at 60 days post breeding are much lower than the fertilization rates quoted above. These range from 20-84.5 % (Boyd et al., 1969; Diskin and Sreenan, 1980; Roche et al., 1981). It has been postulated that this disparity between the expected and observed level of fertility stems mainly from the loss of embryos or fetuses prior to term (Hammond, 1921; Corner, 1923). Embryonic mortality alluded to in this thesis, strictly interpreted, refers exclusively to losses in embryos that are experienced during the period extending from conception to the completion of the stage of differentiation (Committee on Reproductive Nomenclature, 1972). This stage of development in the cow is taken to occur around day 45 post breeding. A recent survey undertaken in the Fraser Valley of British Columbia over the past two years estimated a 48.3 % pregnancy rate. Assuming a 10 % rate of fertilization failure that has been reported to occur in the cow

(Sreenan and Diskin, 1985) this implied a 41.7 % incidence of early embryonic mortality (Rajamahendran et al., 1990).

This study utilized low milk P4 concentrations between days 19 to 21 following breeding as being indicative of a return to oestrus. It should be appreciated, therefore, that such an estimate has a tendency to over estimate the actual level of early embryonic mortality. Such a conclusion is based on the fact that the milk P4 method does not eliminate losses incurred due to anovulation or fertilization failure (Moore, 1985).

Embryonic mortality has for long been known to occur in almost all mammalian species (Boyd, 1965; Hanly, 1961; Short, 1979). The extent of this wastage is greatly influenced by the time at which losses in embryos are estimated. Counting CL has been used as one means of estimating ovulation rates and based on which embryonic losses are also assessed (Ayalon, 1978). Utilizing such a method, Ayalon (1978) estimated the incidence of embryonic mortality to range from 25-40 %. Boyd et al., (1969) reported an 8 % loss by day 25 post breeding, while Roche et al., (1981) estimated the loss at 23 % also taking the estimates at day 25 post breeding. At day 42 post breeding, Ayalon et al., (1978) as well as Diskin and Sreenan, (1980) found the incidence to be 20 and 42 %, respectively. On the other hand, a 5-8 % loss in embryos was reported by Boyd et al., (1969) who took the measurement at 42 days following breeding.

Although it has been suggested that most embryos die between conception and 25-30 days of gestation (Robinson, 1951; Moore et al., 1960; Perry and Rowlands, 1962; Roche et al., 1981; Sreenan and Diskin, 1983), evidence generated over the last 5-6 years indicates that 75-80 % of these losses take place between days 15-

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18 post breeding (Sreenan and Diskin, 1985) ie at the time of placental attachment (King et al., 1982). The majority of cows losing their pregnancies prior to day 16 return to oestrus at day 21 with no indication of the fact that they were pregnant. It has been shown that animals losing their pregnancies on or after day 16, have a slight extension in the oestrous cycle length (Northey and French, 1980; Heyman et al., 1984). This difference due to the fact that day 14-16 has been established to be the time of maternal recognition of pregnancy in the cow (Thatcher et al., 1989).

This loss in embryos early in gestation, has been reported to cost the beef and dairy industries of the United Kingdom alone amounts well in excess of £ 300 million per annum (Flint et al., 1990). This does not seem to be anywhere near what Bishop (1964) referred to as "...losses in embryos...at a low biological cost...".

2.2. Factors Predisposing Early Embryonic Mortality

Embryonic mortality was postulated to be nature's way of eliminating impaired genotypes early in gestation (Bishop, 1964). These genetic abnormalities could either be of maternal or paternal origin, and tend to favour the elimination of any embryos bearing grossly abnormal chromosome complements (Bishop, 1964; Berepubo and Long, 1983). The extent of the losses incurred cannot be accounted for in full solely by genetic abnormalities (Edey, 1969; Gayerie, 1983; Moore, 1985). Various other factors have since been postulated to be involved in the demise of embryos.

It has been reported that in cattle and sheep, twinning rates are lower in unilateral double ovulaters compared to bilateral

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double ovulaters (Hafez, 1976; White et al., 1981; Woods and Ginther, 1983). This indicates that multiple embryos contained in the same horn (ipsilateral) are more liable to be lost compared to those contained in separate horns (contralateral). Similarly, in the human being occasionally, twins embryos do not result in the birth of baby twins. This has been attributed to the disappearance of one of the pair (Landy et al., 1982). The pronghorn antelope and South African long-eared elephant shrew are two animal species that ovulate and fertilize several ova but only give birth to a substantially reduced number of off-spring (Mossman and Duke, 1973).

The presence of pathogens in the uterus has also been shown to increase the basal level of embryonic mortality (Bouters, 1985). The predisposing factors for some of these uterine infections could conditions such as retained placentae (Erb et al., 1958) or prolapse of the reproductive tract (Woodward and Quesenberry, 1956). Retained placentae in dairy cattle can depress pregnancy rates by as much as 40 % (Erb et al., 1958; Patterson et al., 1981). It has also been reported that spontaneous asynchrony between the embryo and the uterus (Rowson and Moor, 1966; Wilmut et al., 1985; Willingham et al., 1986), may also lead to the termination of a pregnancy. The same authors suggested that the phenomenon of asynchrony between the embryo and the uterine milieu could develop spontaneously.

Adverse environmental conditions such as high ambient temperature have been reported to increase the incidence of embryonic mortality by acting directly on the embryo (Alliston et al., 1965; Edey, 1979). Alternatively, high ambient temperature may

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also increase early embryonic mortality by altering the hormonal status of the dam (Thatcher et al., 1974). However, for this deleterious factor to take effect, the body temperature of the dam must increase (Ulberg and Burfening, 1967).

An intrinsic development programme has been postulated to exist in the bovine embryo. The programme assumes that embryos, in the first 3-4 days of their existence, continue growing oblivious of maternal or environmental control. Disruption of this intrinsic programme for development (Johnson, 1979) has been associated with failure of the embryo's ability to communicate with the dam (Bazer and First, 1983). This, in turn, has been implicated in the loss of embryos early in gestation. The loss of communication with the dam is indispensable for normal establishment and maintenance of pregnancy. The process involves the elaboration of factors that either serve as antiluteolysins or as luteotropins. The latter group of products are associated with augmentation of CL synthesis and secretion of P_4.

The quality of the feed that an animal is dependent on has been shown to influence fertility. Nutrition, however, exerts its effects on fertility mostly prior to puberty, around the period of breeding and at parturition (Morrow, 1980). Hence elucidating the mechanism by which nutrition influences reproduction becomes very difficult. Excess dietary protein has been shown to increase the incidence of embryonic mortality (Rattray, 1977). Short and Bellows (1971) found that fewer heifers were bred, fewer pregnancies established and still fewer pregnancies maintained during early pregnancy when animals were at lower rates of gain. Anderson and Melamphy in 1972, reported that higher energy levels in the diet

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resulted in concomitant increase in both ovulation rates and embryonic mortality. Conversely, Dyck and Strain (1979) could not show any such effect in the gilt.

Severe undernutrition and stress (Edey, 1969) have also been shown to increase the incidence of embryonic mortality. Deficiency in dietary constituents such as minerals (e.g. selenium) has been associated with reproductive failure. Inadequate selenium in the diet predisposes the retention of placentae at parturition (Trinder and Renton, 1973). This in turn, renders the uterus susceptible to infection which in turn reduces the chances of an animal establishing and maintaining its pregnancy (Bouters, 1985).

The necessity for the CL in the establishment and maintenance of early pregnancy in domestic animals is well documented (Henricks et al., 1970; Randel et al., 1971; Erb et al., 1976; Bulman and Lamming, 1978). The endocrine function of the ovary was discovered from surgical procedures involving ovariectomy or luteectomy. In either case provision of P_4 from an alternative source was found to maintain pregnancies. Conducting the same experiments but without P_4 replacement resulted in the interruption of pregnancy (Fraenkel, 1903; Amorosso and Perry, 1977). The requirement for this endocrine gland (CL) in the cow, sow, goat and ewe has been established (Hansel, 1988, review). Given the above facts and observations made regarding the high prevalence of low P_4 profiles among animals which fail to maintain their pregnancies, a possible contribution of a dysfunctional CL as a causal agent has attracted considerable attention.

This luteal phase insufficiency is defined as a luteal phase of normal duration but showing reduced secretion of P_4 by the CL of

a specific oestrous cycle (diZerega and Hodgen, 1981). It has been reported that there are, indeed, low P_4 levels during early and/or late luteal phase of the oestrous cycle in non-fertile breedings. This phenomenon is suggestive of an inadequate CL function which has been suspected to occur in cattle (Henricks et al., 1970; Randel et al., 1971; Erb et al., 1976; Bulman and Lamming, 1978; Lukaszewska and Hansel, 1980; Wilmut et al., 1985). Low levels of circulating P_4 have been associated with infertility in various species of livestock (Bulman and Lamming, 1978). Attempts have been made to try and associate concentrations of P_4 preceding a particular AI (Corah et al., 1974; Folman et al., 1973) with the incidence of unsuccessful pregnancies. Folman et al., (1973) found a positive correlation between P_4 levels at 2, 3, 5, 7, 8, 11, 12 and 15 days preceding a particular AI and the prevailing conception rates. Similar results were also reported by Holness et al., (1977) who found higher milk P_4 concentrations on days 7 to 9 prior to a fertile insemination. Rosenberg et al., (1977), on the other hand, found that P_4 levels only reached peak near the time of AI among animals conceiving to that insemination. Contrary to these observations, however, Bulman and Lamming, (1978) found no relationship between pre-service P_4 concentrations and conception rates.

It has been postulated that such low levels of P_4 lead to the impairment (decrease) in the rate at which accelerated transport of the embryo into the uterus takes place (Day and Polge, 1968; Hunter, 1980). Once exposed to such P_4 deficient environments, embryos fail to develop to the blastocyst stage in the pig (Murray et al., 1971). Similarly, in the mouse (Kirby, 1962), the rabbit

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(Adams, 1958), the rat (Alden, 1942) and sheep (Wintenberger-Torres and Flechon, 1974), it has been reported that the capability of these blastocysts to sustain subsequent growth is impaired. This phenomenon is observed despite the fact that morulae will develop into the blastocyst stage in the aforementioned species.

Available evidence indicates that there exists a divergence in peripheral plasma P_4 concentrations in pregnant versus non-fertile inseminations or sham-inseminated cycling cows and that this difference is evident as early as day 6 through day 14-18 (Henricks et al., 1970; Lukaszewska et al., 1979; Hansel, 1981). Using rates of return to oestrus, Fonseca et al., (1983) found that conception rates at first AI postpartum increased in proportion to peripheral concentration of P_4 at 12 days prior to that insemination. It has also been reported that mean concentrations of plasma P_4, at 48-34 hours prior to the pre-ovulatory LH surge, were higher in cows that conceived than in non-fertile inseminations (Erb et al., 1976). The same authors also observed that in animals that conceived, P_4 concentrations were higher at 6 days following AI. This clearly points to an effect of a dysfunctional CL wherever P_4 levels were low.

It is not clear, however, which single factor is responsible for the losses in embryos early in gestation (Heap, 1985). It could just be that these factors vary depending on the locality, the level of management and the environment prevailing at any one given locality.

2.3. Role Of Progesterone In Gestation

The establishment and maintenance of early pregnancy in most mammals has been demonstrated to be dependent on P_4 originating from the ovary (Randel et al., 1971; Erb et al., 1976; Bulman and Lamming, 1978). In mid-gestation (second one half), the involvement of the placenta in P_4 production (luteoplacental shift) ensures the maintenance of pregnancy in most domestic animals except the sow where the presence of an intact and fully functional CL is required throughout gestation (Csapo, 1969).

The levels of the hormone must also remain relatively elevated in order for pregnancy to be maintained (Robinson et al., 1989; Heap, 1972). P4 is also essential for the establishment and maintenance of a quiescent uterus during pregnancy, thus permitting implantation to continue. The secretions of the endometrial secretory granules and hence the uterine milieu are all regulated by P_4 (Flint et al., 1990). Such a mode of uterine milieu control occurring prior to oestrus, serves to prevent the establishment of an environment hostile to the embryo (Moore, 1985).

In addition to the direct effect that P_4 exerts on pregnancy, treatment with P_4 prior to breeding was found to raise the efficiency of oestrus expression in cows from 54 to 71 % (Stevenson et al., 1989). It is also essential for the accelerated transport of embryos from the oviduct into the uterus (Day and Polge, 1968; Hunter, 1980). P4 is involved in the regulation of the secretions of the anterior pituitary gonadotropin, LH (Robinson et al., 1989; Ireland and Roche, 1982; Goodman and Karsch, 1980). LH, on the other hand, is essential not only for the optimal function of CL,

but also the growth and maturation of ovarian follicles. These follicles have, in turn, been suspected of playing a role in CL function. Lukaszewska and Hansel (1980) showed that both P_4 and Oestradiol-17ß (E_2) were higher in pregnant compared to cyclic non-pregnant animals. While levels of P_4 were high between days 10-18, levels of E_2, on the other hand, were high between days 6-16 post oestrus. This was assumed to implicate ovarian follicles in CL function.

2.4. Methods For Reducing Early Embryonic Mortality

The majority of hypotheses developed for the purpose of addressing the problems imposed by an inadequate CL have mostly evolved around trying the following options, viz;

(i) since the assumption is that the CL is not functioning optimally, exogenous P_4 has been provided in order to supplement endogenous P_4 secretion.

(ii) believing that a bovine embryo does influence the secretory capacity of the CL, various laboratories have appropriately concentrated on infusing into the uterus of the cow either viable day 16 embryos or homogenates of embryonic vesicles,

(iii) believing, also that CL function is dependent on continued support from the anterior pituitary secretory products, namely LH, gonadotropins, e.g. human chorionic gonadotropin (hCG) have duly been utilized,

(iv) since the pituitary is stimulated by secretions originating from higher centres (hypothalamus), an alternative rationale has involved the use of gonadotropin releasing hormone (GnRH).

2.4.1. Hormone Replacement Therapy

Exogenous administration of P_4 in ovariectomized animals has been shown to maintain pregnancy to term (Fraenkel, 1903; Amorosso and Perry, 1977; Flint et al., 1990). This clearly emphasised the indispensability of the hormone P_4 in gestation. For this reason, P_4 has been administered in farm animals in an attempt to try and increase circulating levels of P_4 such that levels are elevated to those characteristic of mid-luteal phase levels.

The use of P_4 replacement regimen has, however, had variable effects. In studies utilizing intact animals, peripheral levels of P_4 were increased in some trials (Northey et al., 1985; Robinson et al., 1989). Conversely, there were also no detectable differences in P_4 concentrations between cows given PRIDs either between days 5-12 or days 10-17 post AI. Despite the absence of significant differences at 8 days post AI, there was observed a trend towards higher P_4 values in cows treated with the PRIDs. Endogenous P_4 production was attenuated in animals receiving the treatment between days 10-17 (Robinson et al., 1989). However, such an effect of P_4 treatment was not evident in the trials of Loy et al., (1960) or Zimbelman et al., (1959).

It was against such a background that Walton et al., (1989) concluded that exogenous P_4 supplementation may not be a fruitful approach for overcoming luteal inadequacy in the cow.

2.4.2. Uterine Infusions

In the bovine, only embryos in excess of or equal to 16 days of age have been shown to be luteotropic. Hence one viable option for extending the duration of the luteal phase and enhancing CL

function in the cow has been the use of embryonic vesicles or products elaborated by embryos. Proof of the luteotropic effect of the bovine embryo has since been documented (Betteridge et al., 1980). These authors transferred viable day 16 embryos into recipient cows that had been synchronized to be 16 days post oestrus on the day of transfer. The result was maintenance of CL function and establishment of normal pregnancy. Similarly, it was shown that removing day 17-19 embryos results in the extension of the CL lifespan (Northey and French, 1980). The same result could not be shown in unmated control or in animals from which embryos were removed prior to day 15 post breeding. This confirmed earlier opinions stating that day 16 embryos secrete products which influence the effect of luteolytic agent on the CL and augment endogenous P_4 secretion. These secretory products can either be anti-luteolytic or luteotrophic in function.

Uterine infusions of day 16 embryonic vesicles have since been demonstrated to extend the oestrous cycle in the cow (Betteridge et al., 1980; Northey and French, 1980; Heyman et al., 1984). Conceptually, this enables an embryo that is possibly lagging in development, and hence potentially incapable of preventing luteolysis, to continue growing until it is capable of secreting anti-luteolytic or luteotrophic products which, in turn, permit the establishment of pregnancy (MacCracken et al., 1984).

Both homogenates and aqueous extracts of the day 16-18 blastocysts have been shown to increase net P_4 production by dispersed luteal cells in culture (Beal et al., 1981). The same products could also be deemed as a means for amplifying the signal (bovine trophoblastic protein 1 or bTP-1 in short) elaborated by

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the embryo for the purpose of alerting the dam of the presence of a conceptus in-utero (Thatcher et al., 1989).

Clearly, although this method has been shown to increase the duration of the luteal phase and an increase in the synthesis and secretion of P_4, its application in the field pauses technical problems of its own.

2.4.3. Use Of Gonadotropins

Alternatively, luteotrophic substances such as hCG have been used in an attempt to augment the function of spontaneously derived CL (Christie et al., 1979; Holness, et al., 1982; Santoz-Valadez et al., 1982; Sreenan et al., 1979; Veenhuizen et al., 1972; Wagner et al., 1973). hCG itself, is a glycoprotein whose beta sub-unit bears 90 % homology with the corresponding sub-unit of LH. It is this beta sub-unit that imparts these hormones with their respective biological activity. Due to this homology, hCG has been shown to mimics the actions and effects of LH (Flint et al., 1990).

Although hCG has been shown to improve luteal function, and to increase peripheral P_4 levels, its effect on pregnancy rates remains controversial (Diskin and Sreenan, 1985). Some reports indicate no beneficial effect on pregnancy rates resulting from using hCG (Holness et al., 1982; Sreenan et al., 1979; Wiltbank et al., 1961), other workers have shown only a slight increase in pregnancy rates among treated animals (Santoz-Valadez, et al., 1982) or no beneficial effect at all (Looney et al., 1984).

Walton et al., (1989) treated cows with hCG (1500 IU) on day 5 post insemination and reported a significant difference in both plasma and milk P_4 profiles between hCG treated and control cows.

These significant differences were noted after day 8 for plasma and at days 18 and 20 for milk samples. Treatment with hCG as a method of increasing peripheral levels of P_4 was found to be beneficial in this case. They, however, noted a 3-day extension in the oestrous cycle length in non-fertile inseminations. Wiltbank et. al., (1961) used 1000 IU of hCG given as daily intramuscular injections starting at day 14 until 34. They reported a 6 % improvement in pregnancy rates in cows receiving the treatment.

Wagner et al. (1973) gave 2000 IU of hCG on day 3 and reported an 11 % improvement in pregnancy rates. Santos-Valadez et al., (1982) administered 5000 IU of hCG on day 15 and reported an 11 % increase in pregnancy rates. In a similar experiment, Massey et al. (1983) administered the same dose 7 days after embryo transfer and found a 5.7 % improvement in pregnancy rates. Greve and Lehn-Jensen (1982) treated cows with 1500 IU on alternate days starting on day 13 until day 35 post breeding. Although not significant, hCG treatments increased pregnancy rates by 6 %. Echternkamp and Maurer, (1983) administered 1000 IU of hCG on day 0 and found a 10.2 % reduction in pregnancy rates. When the treatment was given to twice open cows, a 5.5 % loss in pregnancy rates was detected among cows, but not heifers, treated in a similar manner. Sreenan and Diskin, (1983) administered 1500 IU of hCG between day 10 and 20 post oestrus in two trials. They reported a decrease (11 %) in one trial, and increase (11 %) in another.

McDermont et al., (1986) reported giving, in one instance, 3300 IU of hCG to cows of low fertility at day 15. They found hCG could lead to a slight (5.3 %) improvement in pregnancy rates. In another instance, they administered 3000 IU of hCG to animals of

high fertility. They found a slight (6.4 %) decrease in pregnancy rates amongst animals receiving hCG.

While it is clear that the results obtained with the use of hCG in cows have been very variable, it is also quite apparent that we do not seem to be consistent with respect to the dose of hCG administered to animals. The frequency of administering hCG has been as variable as the timing of administering these treatments. One realises and appreciates that the main rationale behind the administration of hCG, at least until now, has mainly been due to the luteotropic effect of hCG.

2.4.4. Use of Gonadotropin Releasing Hormone

Pituitary function is under the control of the hypothalamic decapeptide gonadotropin releasing hormone (GnRH) (Henderson, 1979). Once GnRH is released or administered exogenously, it causes the secretion of the gonadotropins from the anterior pituitary (Kesler et al., 1978). This effect has, however, been shown to be limited to selectively inducing the secretion of LH within approximately 2 hours of exposure to GnRH (Cantley et al., 1975).

Administration of GnRH has been used with the aim of inducing the release of LH from the anterior pituitary. Hence, GnRH has also been used in attempts at improving pregnancy rates in cattle (Lee et al., 1985). It is apparent, however, that the use of GnRH has produced very variable results (Lewis et al., 1990). Nakao et al., (1983) administered 100 μg intramuscularly to cows at the first breeding post partum. They found benefit from using GnRH at breeding on fertility, more so in areas where fertility was already low. In concurrence with these findings, Phatak et al., (1986)

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found a 9.3 % increase in conception rates due to 111 IU of GnRH treatments administered immediately after the fourth breeding. Similar results were also reported by Lee et al., (1983) but different from those reported by Pennington et al., (1985). Echternkamp and Maurer, (1983) administered GnRH on day 0 (day of breeding) and found a 30 % reduction in pregnancy rates among twice-open cows treated with GnRH on day 0.

A series of four experiments conducted by Macmillan et al., (1986) revealed that a single injection with GnRH given between days 1-13 induced different responses in recipient animals depending on the time the treatment was given. Animals receiving 5 μg/hr of GnRH on days 1-6 after the first oestrus lead to a reduction in pregnancy rates. When treatments were administered between days 7-10 and increasing the dose of GnRH to 10 μg, GnRH had no effect on pregnancy rates. Both dosages when given after day 6, however, resulted in increased the number of animals showing extended cycles. This effect was more pronounced in animals receiving the treatments between days 11-13 after the first oestrus post partum. Treatments at a dose of 10 μg rendered between days 11-13 also lead to increases in pregnancy rates in both first and second inseminations. Lee et al., (1985) reported treating 24 dairy cows with 100 μg of GnRH intramuscularly on day 0. Such a treatment lead to significant increases in P4 concentrations during the 4 days after breeding. Pregnancy rates, however, were not influenced by the GnRH treatment.

Examining the literature pertaining to the use of GnRH in cattle leaves one to draw one or two deductions. It appears that GnRH has been used mostly around oestrus with the aim of

synchronizing ovulations. This has found wider application among animals returning for breeding more than 3 times. Such cows have been termed "repeat breeders". Despite showing slight increases in pregnancy rates following the use of GnRH, results obtained to date still remain inconsistent.

2.5. Follicular dynamics in the cow

It was postulated by Rajakoski in 1960 that ovarian follicles in the cow exist as waves of continuous follicular growth. Other workers (Choudry, 1968; Savio et al., 1988; Sirois and Fortune, 1988) have since shown that follicles do indeed exist as continuous waves of (2 or 3) follicular growth. It appears, on one hand, that 80-81 % of heifers tend to have 3 waves of follicular growth while pluriparous cows, on the other hand, normally exhibit 2 waves of follicular growth (Pierson and Ginther, 1986).

Investigations carried out in the UBC laboratories using ultrasound scanning confirmed the existence of waves of follicular growth, leaning towards 2 as being the norm in both cyclic non-pregnant and pregnant dairy cows. A few cows will show 3 waves of follicular growth, but these tend to have extended cycles (Taylor and Rajamahendran, 1990). These waves of follicular growth that have been reported to exist in the non-pregnant cow, have also been determined to be present during early pregnancy (Taylor and Rajamahendran, 1990). These authors also determined that these waves culminate in the emergence of at least one preovulatory-size follicle at days 7 and on day 0. Another pre-ovulatory size follicle may occasionally appear 14 days post breeding but does not seem to reach maximal size and instead regresses paving the way for

a third wave which appears about day 18-19 (Taylor and Rajamahendran, 1990). The follicle ranges from 10-30 mm in diameter. Using ultrasonography, no visual differences were found when the preovulatory-size follicle of the luteal phase and that found at oestrus were compared.

Follicles have been reported to acquire receptors for LH as they enter the antral stage of development (Ireland, 1987). Ireland and Roche (1983) have in fact reported that the follicles present during the oestrous cycle of the cow, have receptors for LH, being maximal on day 7 and a second peak occurs around days 12 to 14 post oestrus. Similar results were reported by Bennett et al., (1989), whose in-vitro and in-vivo studies revealed a greater response to hCG treatment at days 3-7 (early luteal phase) compared to days 8-13 (mid to late luteal phase).

The presence of follicles during the luteal phase and early pregnancy and fully equipped with receptors to LH, affords us with a tool that might have potential for supplementing P_4 levels in the dairy cow. The advent of ultrasound scanning in the early 1970's presented us with an equally powerful aid with which to conduct studies in reproductive physiology. This permits us to visualize the reproductive tract and to obtain real-time or dynamic images of the same (Pierson et al., 1988). This makes it possible to closely follow ovarian dynamics.

3. OBJECTIVES AND RATIONALE

This study was, therefore, aimed at evaluating the efficacy of using hCG for inducing CL development, increasing the size of the spontaneously derived CL and their capability to synthesize and

secrete P4. It was intended also to investigate the effect of such increased P4 concentrations on pregnancy rates in the Holstein breed of dairy cattle.

3.1. OBJECTIVES

3.1.1. Experiment one

The objectives for experiment one, of this study, were to determine if giving hCG either at the time of breeding (oestrus=day 0), 7 or 14 days post breeding would;

(a) Increase the number of CL,

(b) Increase the diameter of the spontaneous CL,

(c) Increase plasma and milk P_4 levels

3.1.2. Experiment two

The objectives of experiment two were to;

(1) increase milk P_4 levels in hCG treated animals.

(2) reduce the extent of embryonic mortality by reducing that which is caused by CL dysfunction.

3.2. RATIONALE

The aspirations borne during the implementation of this experiment, were as follows;

3.2.1. Day 0 (oestrus)

(i) Giving hCG at the time of breeding would ensure ovulation of the dominant follicle present at that time.

(ii) hCG given at the time of breeding would possibly influence the formation, growth and function of the CL.

3.2.2. Day 7 post oestrus

(i)   Treatment with hCG at 7 days post breeding would attempt to ovulate the dominant follicle present on that day.

(ii) To increase both the size and function of the spontaneously derived CL.

3.2.3. Day 14 post oestrus

(i)   hCG was administered on day 14 post breeding so as to ovulate the dominant follicle present at this stage of the luteal phase.

(ii) It was also intended that hCG injection given at day 14 post breeding would augment a possibly waning endogenous P_4 production. This was deemed to be a critical time in the life span of the CL as this represents the time when the CL begins its exposure to the influence of luteolytic prostaglandins of uterine origin. It also represents the timing of signal transmission from the embryo to the dam, thus initiating the process of maternal recognition of pregnancy. Supplementing P4 production at this time would, conceptually, allow the embryo some extra time for signalling its presence.

4. Experiment One: Effect Of hCG Administration At Various Times Following Breeding On CL Number, Diameter And Progesterone Profiles In Dairy Cattle.

4.1. MATERIALS AND METHODS

4.1.1. Animals : General Management Practices

When this experiment was conducted, the UBC farm at South Campus had almost 100 Holstein dairy cows, 47 of which were milking. The cows were housed in free stall barns. Cows seen in oestrus between September, 1989 and May, 1990 were included in the trial. Cows used in the experiment ranged from 3-8 years of age and were maintained on a diet consisting of a dairy textured concentrate, alfalfa cubes (both 16 % protein), chopped grass (11 % protein). The dairy concentrate ration contained grains (corn, barley, oats), soybean and mineral supplements. In addition to grain mixtures, lactating cows were allowed free access to an iodized cobalt salt block lick. All milking cows were fed on average a production ration of 1 part of feed for every 3-4 kg of milk produced. The feed to milk ratio was dependent on the stage of lactation that each cow was at. Dry cows, on the other hand, were allocated daily 10 kg of hay and 12 kg of alfalfa cubes. The cows were examined after parturition by a veterinarian from the Animal Care Centre. The first breeding post partum were conducted after day 60. Only cows confirmed to have resumed cycling were used in the experiment. During the period of conducting the experiment, the average milk production was 7802 kg, with 2.90 % protein and 3.20 % butter fat.

Although detection of oestrus was conducted by most of the farm workers, a greater number of heat checks were done by the respective milkers on duty. Animals were checked for oestrus at least twice daily, between 02:00 to 04:00 and between 14:00 and 17:00 hours. Oestrus detection was based on observing for cows attempting to mount and standing to be mounted by other cows, presence of vaginal discharge, restlessness, and feeding habits. An attempt was also made to verify the occurrence of unobserved oestrus by noting whether or not there was metoestrus bleeding. When detected, this was assumed to indicate that the animal had been in oestrus within the last 2-3 days. The day of oestrus was designated as day 0 of the oestrous cycle. All cows seen in standing heat were bred approximately 12 hrs later. All breedings were carried out by farm technicians trained in artificial insemination (AI).

4.1.2. Treatments

At unsynchronized oestrus, thirty four lactating dairy cows were randomly assigned to one of four treatment groups. A single intramuscular injection containing 1000 IU of hCG (1 ml of APL^R, hCG, Ayerst Laboratories, Montreal, Canada) was administered either on day 0, (n=8), day 7 (n=9), 14 (n=9) post breeding, or no hCG treatment (control, n=8).

4.1.3. Ultrasound Examination

A real time ultrasound scanning device (Ultrasound scanner, model LS 300, Tokyo Keiki Company Limited, Tokyo, Japan) equipped with a 5 Mega Hertz (MHz) probe was used to monitor ovarian

dynamics during the time of investigation. The 5 MHz transducer was chosen due to its greater resolving power. This capability made it more suitable for examining deeply situated structures such as ovaries. Pierson et al., (1988) described development of real-time ultrasound scanning in the late 1970's as the most profound technological advance in the field of large animal research and clinical reproduction since the introduction of transrectal palpation and radioimmunoassay (RIA) of circulating hormones. Thus, we now have a non-invasive method for visualizing the reproductive organs and their payload-the conceptus".

At each ultrasound evaluation, the rectum was evacuated of all faecal material and a quick note was made of the uterine tone and the location of the ovaries was established by palpating per rectum. The procedure of ultrasound examination was that described by Squires et al., (1988). During ultrasound examination, internal structures in the body of cows fall under two categories. The probe emitted sound waves. The characteristics of specific tissues determined what proportion of the sound beam were reflected. Images of the reflected portions registered on the screen as shades of grey, extending from black to white. All fluid-filled body structures being non-echogenic (absorb ultrasound waves emitted by the probe) appeared black. Follicles for example were visualized as black, roughly circular ultrasound images (plate 1). On the other hand, dense tissues such as bone or cervix appeared white. Tissues in between these extremities were seen as different shades of grey, depending upon their degree of echogenicity (Pierson et al., 1988). In order to keep a permanent record and to facilitate the measurement of the structures of interest, once the optimum scan

was obtained, these were frozen on the screen. The dimensions of CL and follicles measuring more than 3 mm in diameter were obtained. Measurements were taken at the widest poles using a built-in system of calibrated callipers on the ultrasound machine. Information on individual cows was recorded on the frozen screen/images using an alpha-numeric key board. Such data included the date and time of scanning, the cow identification number, number of days post AI. Additional data pertaining to the measurements of the structures of interest were recorded on the same frozen screen. Noted, too, was the tone and contents (where present), of the uterus. Hard copies of the frozen images of the CL and follicles more than 3 mm were obtained using a Mitsubishi Video copy processor (Model number P60U, Mitsubishi Electric Sales America Inc., Cypress, California, USA) connected to the ultrasound scanner.

Ovulations resulting from treatment with hCG were verified by observing for an acute disappearance of one or more of the dominant or pre-ovulatory size (antral) follicles present at the time of administering the treatment. The emergence of an induced CL was characterized by luteal tissue appearing on a site previously occupied by an antral follicle. The growth of such induced CL was followed by determining the diameter of the "new" luteal tissue at the widest poles. This, in some cases, was only possible until the induced CL reached the size of the spontaneous CL. Due to this compounding nature of the CL dimensions, all diametrical measurements were summed up to yield a total diameter of the luteal tissue in each experimental animal. Cows developing accessory CL were palpated per rectum between days 75-80 to determine the fate

of the induced CL and/or the persistence of cystic structures observed following treatment with hCG.

The functional capacity of induced CL could not be established from this _in-vivo_ study. It was, however, hoped that increased levels of circulating P_4 would be indicative of an additive effect of an increase in spontaneous CL production of P_4 and P_4 originating from the induced CL. The compounding nature of the effect of hCG on P_4 production from both the induced and spontaneous CL, were fully appreciated. It was not possible to separate the two sources of P_4.
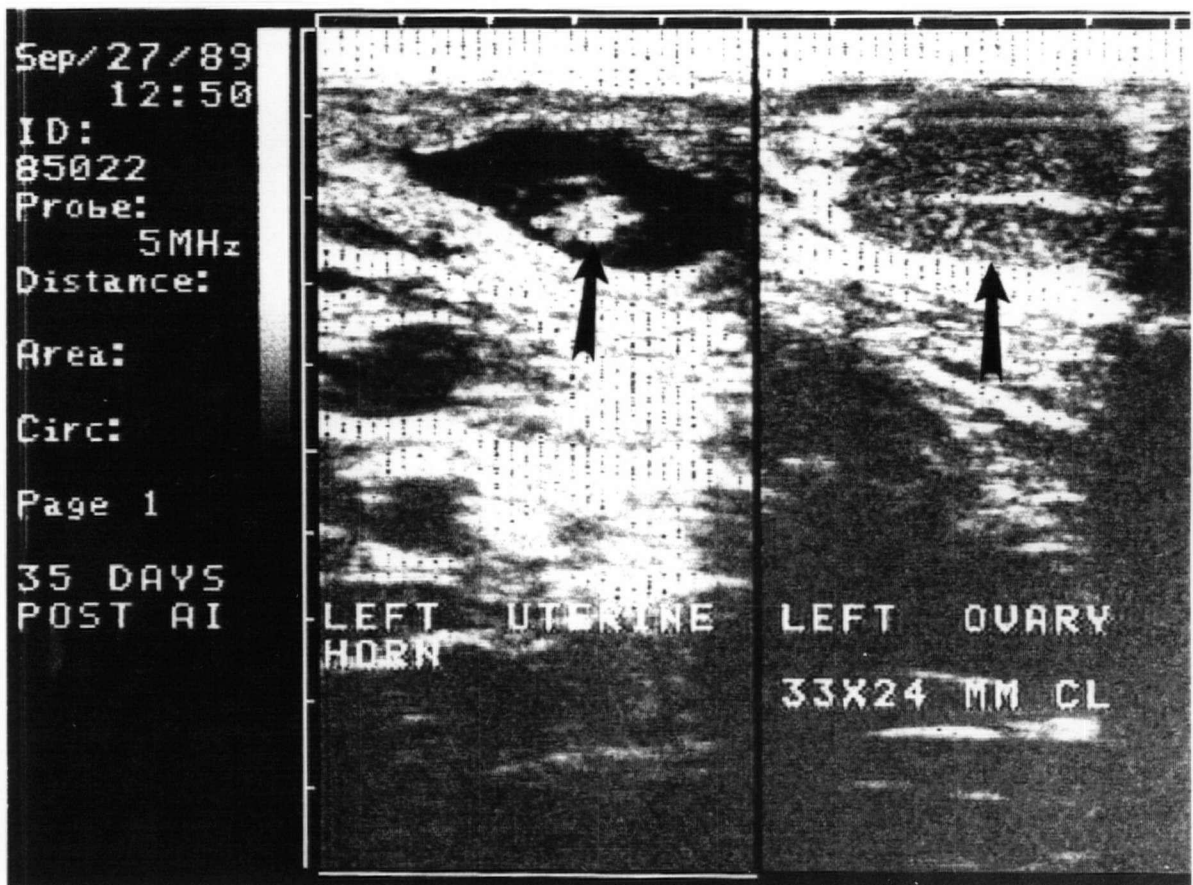
Plate 1. Ultrasound image of a uterine horn and ovary of a cow (85022). Note CL (echogenic) on right ovary (arrow on right screen) and an embryo proper (echogenic) enclosed within embryonic vesicles (arrow on the left screen).

4.1.4. Blood and Milk Sample Collection

At the time of ultrasound evaluation, each animal was bled via the coccygeal vein/artery. 10 ml of blood was collected in vacutainer tubes (Becton Dickinson, Vacutainer Systems, Rutherford, New Jersey, USA) containing sodium heparin. All blood samples were centrifuged within an hour of collection and plasma separated. Blood samples were collected either immediately prior to or after the afternoon milking (15:00 hours) at which time bulk milk samples were collected. All milk samples were collected in 13 dram snap-top sample vials (Capital Vial Corporation, Fonda, New York, USA via Systems Plus). A potassium dichromate preservative tablet (J.R. Dairy Laboratories, Burnaby, B.C., Canada) was dissolved in each milk sample. Both milk and plasma samples were frozen soon after collection and only thawed when assaying for P_4. Ultrasound scanning, milk and blood sample collection were carried out on days 7, 9, 11, 14, 16, 18, 21, 28, 35 and 42 post AI or until the first observed oestrus occurring prior to day 42. All control cows were only scanned and sampled at weekly intervals starting on day 7 post breeding.

4.1.5. Radioimmunoassay (RIA)

The Coat-A-Count[R] P_4 RIA kit (Coat-A-Count[R] Progesterone kit, Diagnostic Products Corporation, Los Angeles, California, USA) procedure used for P_4 analysis was a solid-phase RIA. Over a fixed period of time, radio-labelled P_4 (buffered $^{125}I-P_4$) competes for binding sites with native P_4 in samples. The Coat-A-Count[R] kits consisted of polypropylene tubes coated with antibodies to P_4, iodinated P_4 ($^{125}I-P_4$ or tracer), human serum-based standards having

P_4 values ranging from 0.1 to 40.0 ng/ml (in ready-to-use liquid form). The coat-A-count[R] kit P_4 antiserum used was of a very high specificity for P_4 and low cross reactivity (<10 %) to other steroids which might have been present in the samples.

Three groups of blood samples were obtained from cows either observed to be in oestrus, early dioestrus (days 3-5) or late dioestrus (days 12-14). Samples belonging to each type were pooled together and aliquoted into several tubes. Values from these tubes were used to calculate the inter-assay coefficient of variation (CV). Intra-assay CV, on the other hand, were calculated using the duplicate samples of each standard within an assay. While the intra-assay CV ranged from 1.36 to 7.89, the inter-assay CV for the oestrual, early and late dioestrus samples were 10.3, 11.0 and 9.86, respectively.

At each assay an aliquot of 100 μl of standard, was transferred into the appropriate antibody (Ab) coated tubes (labelled A1, A2, B1,....G1 and G2). Tubes containing standards A through G corresponded to P_4 concentrations of 0.00, 0.1, 0.5, 2, 10, 20 and 40 ng/ml, respectively. A 100 μl aliquot from each sample was transferred into antibody coated tubes and numbered 19....n, where n was the number of the last tube in any given assay. One millilitre (1 ml) of the tracer (^{125}I-P_4) was added into each tube, including two pairs of plain polypropylene tubes. These four plain tubes were included in order to determine the total activity of the tracer (2 tubes), and amount of non-specific binding (2 tubes). After adding the tracer, the tubes were incubated for 3 hrs. After incubation, the tracer was decanted from all tubes, except the total count tubes, and any visible moisture

above the 1 ml line was wiped out from all decanted tubes using cotton swabs (Q-tips[R]). The amount of radioactivity (counts per minute = CPM) bound to antibody in the tubes was quantified in a gamma counter. Using Statistical Analysis System (SAS), logit-log transformation of the standards was performed so as to yield a standard curve which was used to derive the concentrations of P_4 for each respective sample.

4.1.6. Radioimmunoassay Kit Validation.

The concentrations of P_4 in milk and plasma were quantified as described above. While blood plasma has been the main biological material utilized in the assessment of the reproductive status of livestock, the discovery in the 1970's that progestagens were detectable in milk (Darling et al., 1972) pointed to the potential use of such a material in routine diagnostic procedures. It was proposed that progestagen concentrations in milk are one means of diagnosing early pregnancy (Laing and Heap, 1971). A simple and rapid RIA for quantifying "progestagens" in milk soon followed (Heap et al., 1973). RIA in the present experiment was performed using commercially available kits. These kits have since been validated (Gowan and Etches, 1979; Srikandakumar et al., 1986; Robinson et al., 1989) and found to be a reliable tool for monitoring luteal function in the cow as well as other domestic cows (Srikandakumar et al., 1986). Preliminary data from research undertaken in the Department of Animal Science using the Coat-A-Count[R] P_4 RIA kit indicated that P_4 levels lower than 1.00 ng/ml, in both plasma or milk samples, were indicative of the presence of a non-functional CL or complete absence of one (Sianangama and

Rajamahendran, unpublished results, 1989). Such observations were confirmed using ultrasound scanning. The findings regarding the kit are in agreement with those obtained by other laboratories (Srikandakumar et al., 1986; Gowan and Etches, 1986). A P_4 concentration of 1.00 ng/ml was, therefore, used to discriminate between fertile versus non-fertile inseminations.

4.1.7. Pregnancy Diagnosis

Establishment of pregnancy was verified using ultrasonongraphy. Detection of embryonic vesicles was always possible by day 21 post AI. The embryo proper, on the other hand, was consistently identifiable on day 28 post AI. Cows not returning to oestrus by day 42 were palpated per rectum to determine whether they were pregnant or not. The pregnancy check done at day 60 was carried out by a veterinarian.

4.1.8. Statistical Analysis

Data for milk and plasma P_4 and CL diameter were analyzed using a split-plot experimental design. The least square analysis of variance procedure in the General Linear Models, Statistical Analysis System (SAS Institute, Inc., SAS User's Guide: Statistics, 1987, Version 6.3, Edition) was used to test for differences among treatments. The model used was as follows:

$$Y_{ijk} = U + A_i + B_j(A_i) + C_k + A_i \times C_k + E_{ijk}$$

where $Y = n^{th}$ observation, U = population mean, A_i = treatment, $B_j(A_i)$ = cow nested within treatment, C_k = sample number or number of days post breeding (AI), $A_i \times C_k$ = treatment

interaction with number of days post AI, E_{ijk} = experimental (residual) error.

The cow(treatment) mean square was used as error term to test treatment mean square. The other effects ((cow(treatment), sample number and time interaction with treatment), were tested using the residual mean square. Due to variability in the frequency of sampling, each data set was analyzed in two batches (sampling days 7, 14, 21, 28, 35 and 42 and days 9, 11, 16, and 18). Contingency tables in Chi-square analysis was used to test for treatment effect on pregnancy rates. Unless stated otherwise, all comparisons were made at the 5 % level of significance ($P<0.05$).

5. RESULTS

5.1. Induction Of Accessory Corpora Lutea

Results of the responsiveness of cows to hCG treatment are summarized in table 1. Ultrasound scanning of the ovaries revealed that 1/8 control cows had a spontaneous twin ovulation (plate 2b) and 1/8 failed to ovulate (plate 3b). The other 6 had normal CL. The total number of CL in the control group ranged from 0 to 2. Among cows treated on day 0, 3/8 were found to have twin ovulations (plate 2a). At the level of the ultrasound machine it was not possible, in two of the three cows, to establish whether any one of the twin CL were induced by the treatment. Both CL had the typical day 7 appearance, as described above. One of the two CL in the third cow was atypical of that resulting from a spontaneous ovulation. It was small in appearance (9 mm). This particular induced CL, however, regressed by day 21 post oestrus. The spontaneous CL persisted until day 42 post breeding. One cow did

not ovulate and proceeded to develop a cystic follicle. This follicle appeared luteinized as evidenced by a layer of fine luteal-like grainy area circumscribing the dark follicular fluid (plate 3a). For this reason, the P_4 profiles resulting from the luteinized structure, in this cow were not used in the subsequent statistical analysis. Of interest, however, was the fact that the concentrations of P_4 in this particular cow were comparable to those of CL-bearing cows in the control group. The total number of CL per cow among cows treated with hCG on day 0, therefore, ranged from 0 to 2.

Amongst cows treated with hCG on day 7 post AI, the dominant follicle present at the time of administering the treatment ranged in size from 13 to 22 mm in diameter. The second largest follicle, on the other hand, measured 9 to 21 mm diameter. The orientation of the induced CL developed following treatment with hCG did not seem to be influenced by the position of the spontaneous CL. The dominant follicle was the ovulatory follicle in 5/9 cows treated on day 7 post AI. This responsive dominant follicle was positioned either contralateral or ipsilateral (plate 4a) with respect to the spontaneous CL and there were 2 cases of each orientation observed. Plate 4b clearly authenticates the persistence of an induced CL until day 70 when an additional ultrasound evaluation was conducted in cows developing accessory CL. The persistent follicles that appeared either luteinized or showed no obvious signs of luteinization following treatment with hCG on day 7 post AI, were all found to have regressed by day 64 post AI. In the fifth cow, the dominant follicle on one ovary and the second largest follicle on the other ovary, ovulated. This particular cow had double

spontaneous ovulations and these were, therefore, additional two CL induced by the treatment. In 2/9 cows, the second largest follicle located ipsilateral to the CL resulted in an induced ovulation. The largest follicle was present after the emergence of the induced CL but subsequently regressed. Thus, seven out of the nine cows treated on day 7 post AI developed accessory CL. The number of CL per cow among cows receiving treatment with hCG on day 7 post breeding, ranged from 1 to 4.

Among cows treated on day 14 post AI, 4/9 were found to have accessory CL. The dominant follicle present when treatments were administered ranged in size from 9 to 27 mm. In 3/4 of these cows a dominant follicle, located contralateral to the CL, developed an accessory CL. Of these three cases, two were dominant follicles, while the third was the second largest follicle. The only induced ovulation positioned ipsilateral to the spontaneous CL was a 9 mm, third largest but the only growing follicle observed in the fourth cow. Both CL had regressed by day 29 post oestrus. One cow treated on day 14 post breeding was found to have an "apparently" non-luteinized follicle persisting following the hCG treatment. This follicle was shown to have regressed by day 75 post breeding. The total number of CL, per cow in this treatment group, ranged in number from 1 to 2.

Table 1. Ovulatory Response Of Cows Treated With Human Chorionic Gonadotropin Either At The Time Of Breeding, Day 7 or 14 Post Breeding In Comparison To Control Cows.

	Treatment Group				
	Day 0	Day 7	Day 14	Control	Total
Number Of Cows Treated (n)	8	9	9	8	34
Number of Cows Developing Accessory Corpora Lutea	3	7	4	1	15
Number Of Corpora Lutea (Range)/cow	0 - 2	1 - 4	1 - 2	0 - 2	0 - 4

(a)
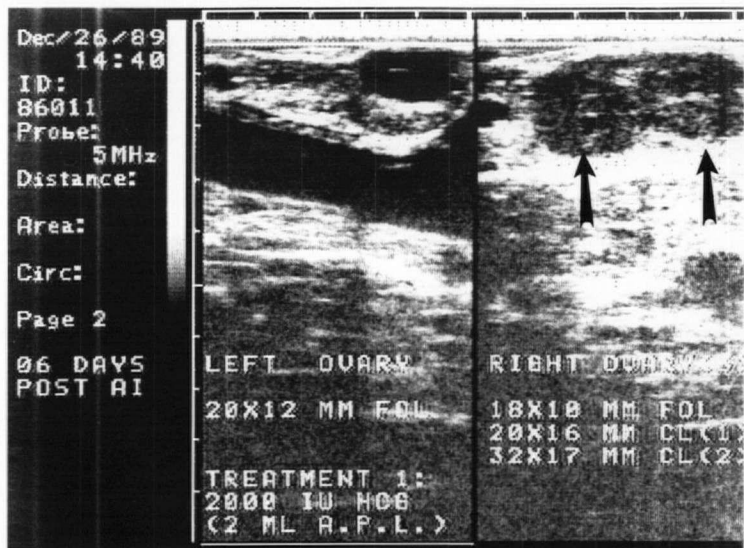
(b)
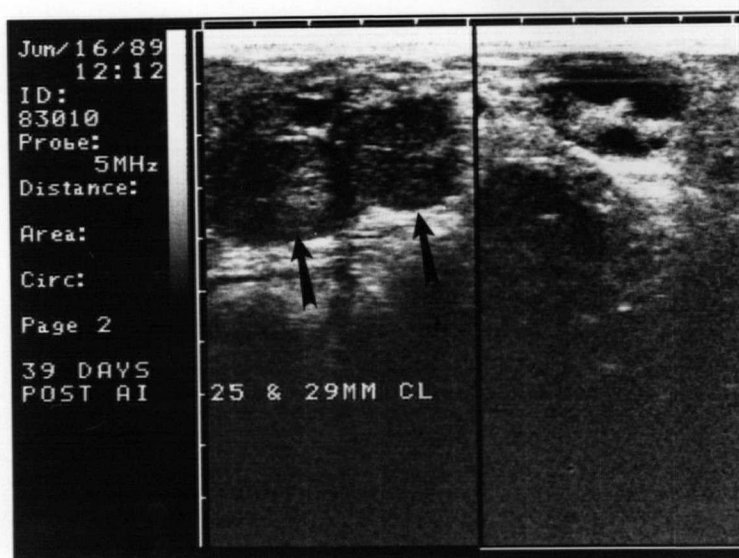
Plate 2. (a) Twin ovulations (arrows on right screen) in animal
             # 86011 treated with HCG on day 0. No apparent
             difference between the two CL.

          (b) Spontaneous twin ovulations (arrow on left screen)
             in animal # 83010.

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(a)
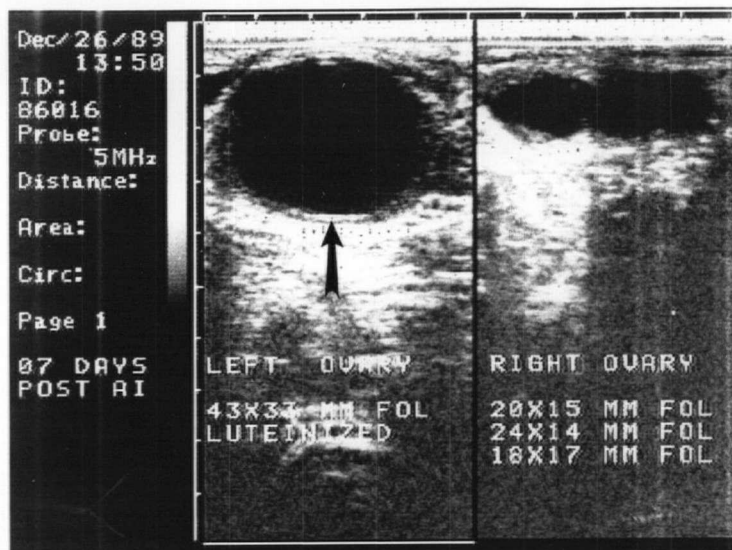
(b)
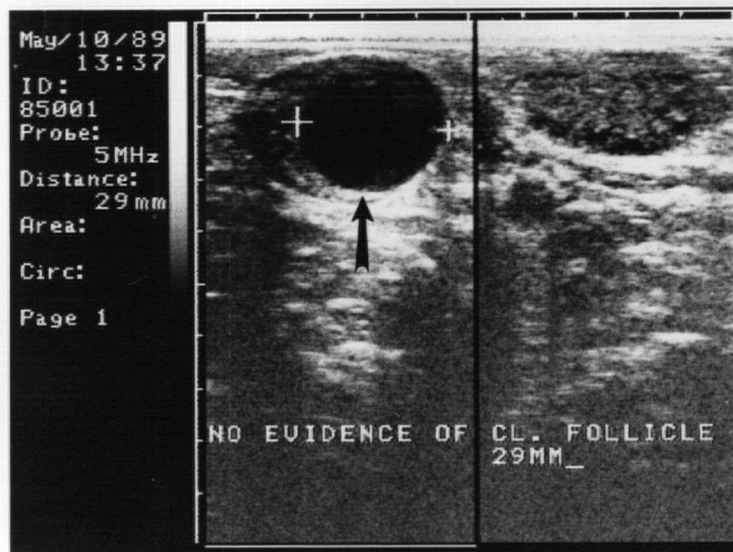
Plate 3.   (a) Cystic follicle (arrow on left screen) in
              animal # 86016 treated with HCG on day 0.

           (b) Cystic follicle (arrow on left screen) in animal
              # 85001 (control).

(a)
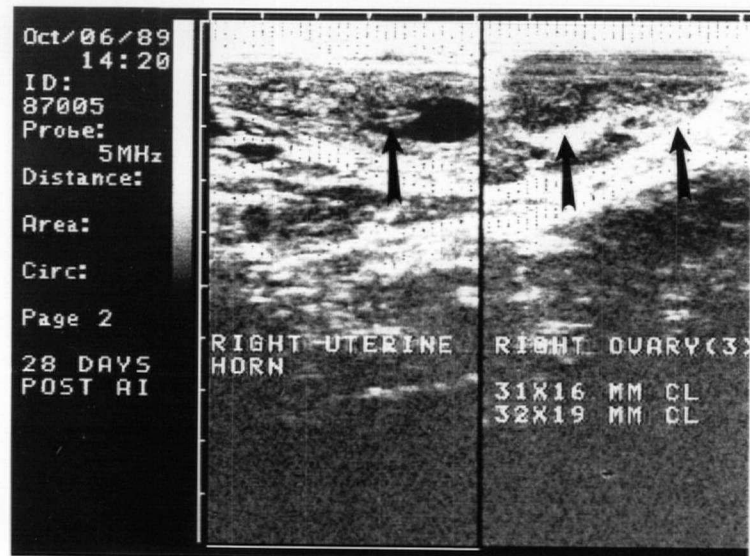
(b)
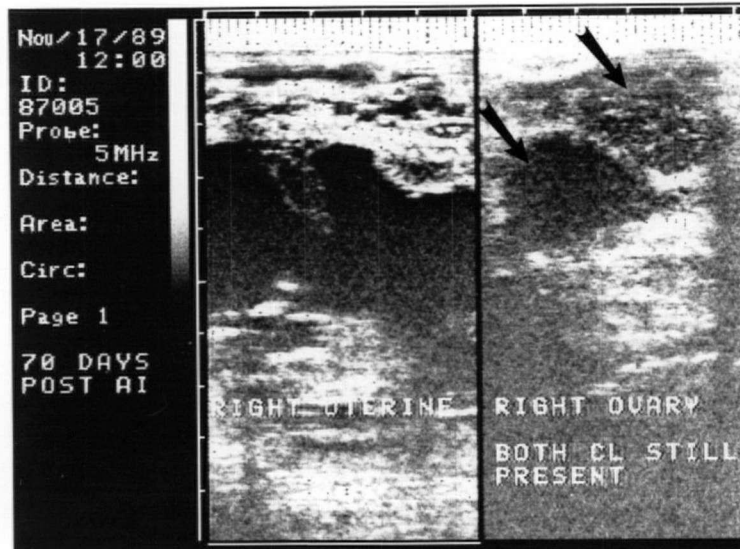
Plate 4. (a) Day 28 images of right ovary (right screen) and
             right uterine horn (left screen). Note both CL
             present (arrows on right screen), and the embryo
             proper (arrow on left screen).

          (b) Day 70 image of right ovary (right screen) and a
             section of the right uterine horn. Note both CL
             are still present (arrows on right screen).

5.2. Growth of The Corpora Lutea

Profiles of CL diameter and LSMeans are presented in figure 1 and table 2, respectively. Results of the analysis of variance of the CL diameter are presented in appendix 1a. Diameter increased significantly ($P<0.02$) with hCG treatment for day 7, 14, 21, 28, 35 and 42 values. Figure 1 show higher CL diameter profiles among cows receiving hCG treatment either on day 0, 7 or 14 post AI compared to the control cows. Overall LSMeans for CL diameter were higher among day 7 treated cows (50.22 ± 2.08 mm) compared to day 0 treated (35.94 ± 2.64 mm), day 14 treated (35.45 ± 2.15 mm) and control cows (29.13 ± 2.60 mm). The overall effect of the sampling periods on CL diameter was not significant. However, overall LSMeans for CL diameter increased from 34.14 ± 2.23 to 39.96 ± 2.28 mm around which level they remained until day 42.

For samples collected on days 9, 11, 16 and 18 post AI the significant interaction between sampling period and treatment made it is difficult to separate the effect of these two effects. The variability between individual cows receiving hCG was also high. Nonetheless, at day 9 post AI, cows treated on day 0 had higher LSMeans for CL diameter than any of the other groups of cows treated subsequently. The LSMeans for the three groups were 45.14 ± 3.10, 38.97 ± 2.98 and 34.56 ± 4.56 mm, for day 0, 7 and 14 treated cows, respectively. By day 11 post AI, LSMeans for CL diameter were, however, significantly higher ($P<0.01$) in cows treated either on day 0 (47.43 ± 3.10 mm) or 7 days post AI (49.47 ± 2.98 mm) compared to cows receiving their treatment on day 14 post AI (34.52 ± 3.35 mm).

At day 16 post AI, both groups of cows receiving treatment either on day 0 (42.70 ± 3.46 mm) or 7 post AI (58.75 ± 2.95 mm) had significantly higher ($P < 0.05$ and $P < 0.01$), respectively) least squares mean values for CL diameter compared to cows receiving treatment on day 14 post AI (33.62 ± 3.06 mm). However, LSMeans for CL diameter of cows treated on day 7 post AI were significantly higher ($P < 0.01$) than cows treated on day 0. At 18 days post AI, cows treated on day 7 (57.67 ± 2.73 mm) still had significantly higher ($P < 0.01$) LSMeans for the CL diameter compared to both groups of cows treated either on day 0 (39.04 ± 3.46 mm) or 14 days post AI (43.05 ± 2.98 mm). There were no significant differences between LSMeans for the CL diameter of cows receiving treatment on day 0 when compared to those treated on day 14 post AI.

Table 2. Effect Of Human Chorionic Gonadotropin Treatment Given Either At The Time Of Breeding, Day 7 or 14 Post Breeding, On Corpus Luteum Diameter* In Holstein Dairy Cows.

Days Post Breeding	Treatment Group[abc]			
	Day 0	Day 7	Day 14	Control
7	42.14 ± 4.53^a	30.75 ± 4.30^b	31.89 ± 4.00^b	28.30 ± 5.02^b
9	45.14 ± 3.10^a	38.97 ± 2.98^b	34.56 ± 4.56^b	
11	47.43 ± 3.10^a	49.47 ± 2.98^a	34.52 ± 3.35^b	
14	35.86 ± 4.54^b	55.00 ± 4.30^a	33.63 ± 4.67^b	32.57 ± 4.54^b
16	42.70 ± 3.46^a	58.75 ± 2.95^b	33.62 ± 3.06^a	
18	39.03 ± 3.46^a	57.67 ± 2.73^b	43.05 ± 2.98^a	
21	33.80 ± 5.74^b	54.20 ± 4.36^a	33.03 ± 4.36^b	30.00 ± 4.54^b
28	34.80 ± 5.74^{bc}	52.23 ± 4.75^a	44.09 ± 5.22^{ab}	26.08 ± 5.62^c
35	36.74 ± 7.49^{ab}	54.22 ± 5.19^a	35.76 ± 5.21^b	27.40 ± 7.47^b
42	31.88 ± 6.45^b	53.25 ± 5.73^a	33.27 ± 5.74^b	30.40 ± 7.47^b

[a,b,c]Numbers with different letter superscripts within row differ at the 5 percent level of confidence (P<0.05.).

* Values are presented as least squares means for CL diameter.

Figure 1. Effects Of Human Chorionic Gonadotropin On The Growth Of The CL In Dairy Cattle (UBC)
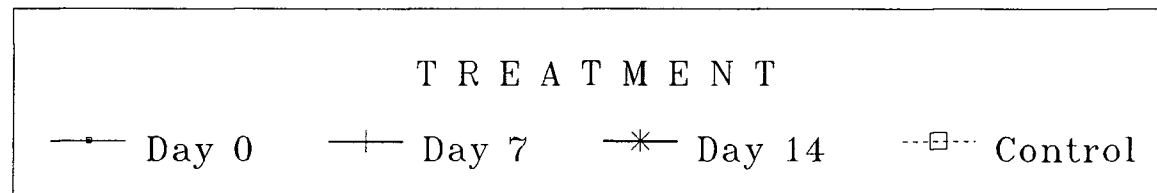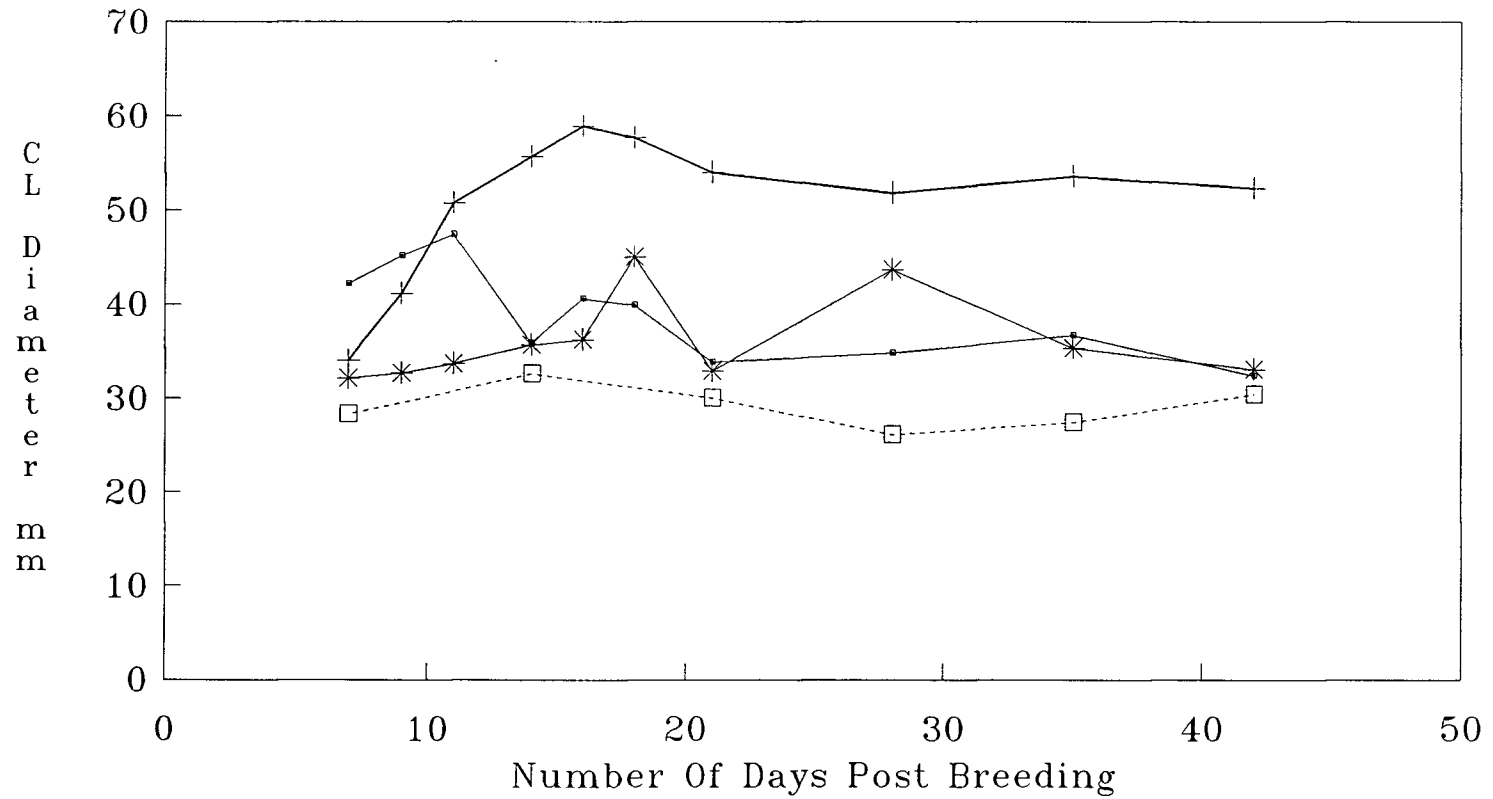
5.3. Progesterone Profiles

5.3.1. Plasma Progesterone

Profiles, LSMeans and analysis of variance on plasma P_4 concentrations are presented in figure 2, table 3 and appendix 2, respectively. Administration of hCG did not lead to significant increase in P_4 at days 7, 14, 21, 28, 35 and 42 post AI. However, there was a trend for higher profiles among cows treated at either day 7 (6.06±0.40 ng/ml) and day 14 (4.73±0.43 ng/ml) in comparison to cows treated on day 0 (3.92±0.62 ng/ml) or control (3.84±0.46 ng/ml). P_4 concentrations were significantly ($P<0.01$) influenced by the sampling period. The response of cows receiving hCG was highly variable. Analysis of samples collected at days 9, 11, 16 or 18 post AI shows no effect of hCG on progesterone profiles. High individual ($P<0.01$) variations among cows receiving hCG are indicated during this sampling period. Overall P_4 increased with time post AI. Levels rose from 4.03± 0.53 ng/ml at day 9 to a high of 6.62±0.54 ng/ml on day 16 but decline thereafter.

By day 18 post AI, both day 7 and 14 treated cows had significantly higher plasma P_4 concentrations compared to cows treated on day 0. The respective LSMeans for plasma P_4 concentrations were 3.51±1.05, 5.97±0.90 and 6.57±0.90 for cows treated on day 0, day 7 or 14 post AI, in that order. At day 35, LSMeans for plasma P_4 concentrations of cows treated on day 7 (9.49±1.02 ng/ml) were not different ($P<0.05$) from those for cows treated on day 0 (4.29±2.58 ng/ml) but were different ($P<0.02$) from those of cows either treated on day 14 (5.61±1.03 ng/ml) or receiving no treatment with hCG (5.72±1.27 ng/ml). Forty two days AI, no differences could be detected in the LSMeans for plasma P_4

among cows treated on day 7 (7.18±1.02 ng/ml) compared to those receiving their treatment on day 14 post AI (4.50±1.13 ng/ml). Cows treated on day 7, however, had LSMeans for plasma P_4 concentrations higher than those treated on day 0 (4.21±1.13 ng/ml) and the control group (1.46±1.27 ng/ml).

Table 3. Effect Of Human Chorionic Gonadotropin Treatment Given Either At The Time Of Breeding, Day 7 or 14 Post Breeding, On Plasma Progesterone Concentrations[*] In Holstein Dairy Cows.

Days Post Breeding	Treatment Group[a,b]			
	Day 0	Day 7	Day 14	Control
7	2.86±0.89	2.40±0.79	2.90±0.85	2.60±0.84
9	3.62±0.94	4.12±0.83	4.37±0.97	
11	4.85±0.94	5.90±0.83	5.23±0.83	
14	4.40±0.85	6.27±0.85	4.90±0.79	4.96±0.84
16	5.27±1.05	7.38±0.90	7.20±0.83	
18	3.51±1.05[a]	5.97±0.90a[b]	6.57±0.90[b]	
21	4.08±1.13	5.42±0.93	5.26±0.93	3.38±0.84
28	3.68±1.13	5.57±0.93	5.20±1.13	4.92±1.12
35	4.29±2.58[ab]	9.49±1.02[a]	5.61±1.03[b]	5.72±1.27[b]
42	4.21±1.13[a]	7.18±1.02[b]	4.50±1.13[ab]	1.47±1.27[a]

[a,b]Numbers with different letter superscripts within a row differ at least at the 5 % (P<0.05) level of confidence.

[*] Values are presented as least squares means for Plasma P_4.

Figure 2. Effect Of Human Chorionic Gonadotropin On Plasma P4 Concentrations In Dairy Cattle (UBC)
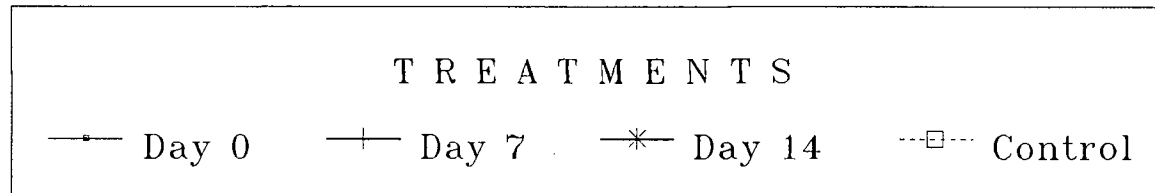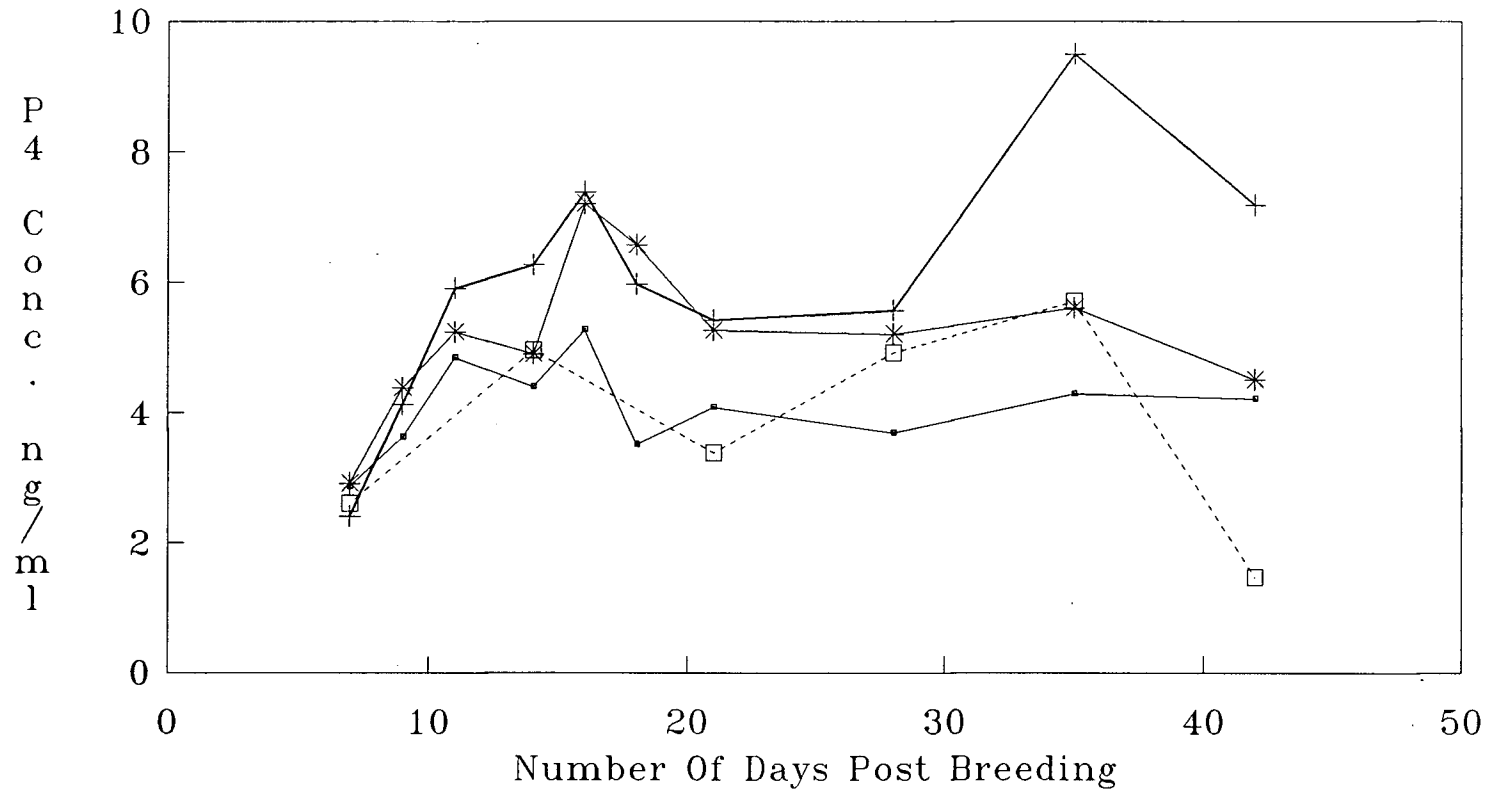
5.3.2. Milk Progesterone

The profiles, LSMeans and analysis of variance on P_4 concentrations are presented in figure 3, table 4 and appendix 3, respectively. Treatment with hCG did not show significant P_4 increase in milk samples collected on days 7, 14, 21, 28, 35 and 42. Sampling period showed significant contribution to P_4 increases. However, individual animal variability was also highly significant ($P < 0.01$). Treatments with hCG lead to significant increase in milk P_4 of samples collected on days 9, 11, 16 and 18. Overall LSMeans during this period were 10.97 ± 0.94, 12.22 ± 0.77 and 24.68 ± 2.25 ng/ml for cows treated either on day 0, 7 or 14. Although the response of cows to hCG treatment was highly significant, there did not seem to be an apparent effect of sampling period. At day 21 post AI, the LSMeans for milk P_4 concentrations among cows receiving treatment with hCG on day 7 (12.60 ± 2.03 ng/ml) were significantly higher compared to those of cows in the control group (7.19 ± 1.98 ng/ml). These were, however, not different from those of cows treated either on day 0 (9.22 ± 2.68 ng/ml) or on day 14 post AI (10.41 ± 2.03 ng/ml).

By 28 days post AI, all cows receiving treatment with hCG had significantly higher milk P_4 concentrations compared to the control group cows (4.94 ± 2.38 ng/ml). There were no detectable differences among cows receiving treatment with hCG. The LSMeans for milk P_4 of cows treated at day 0, 7 and 14 were 15.97 ± 2.68, 12.53 ± 2.21 and 13.17 ± 2.42 ng/ml, respectively. At day 35, cows treated on day 14 (17.76 ± 2.68 ng/ml) had higher LSMeans for milk P_4 compared to cows not receiving treatment with hCG (8.72 ± 2.98 ng/ml). The day 14 treated cows, however, had LSMeans for milk P_4 not different from

those of cows treated either on day 0 (12.68±3.02 ng/ml) or at 7 days post AI (14.86±2.41 ng/ml). Cows receiving hCG either on day 0 or those treated on day 7 post AI, were also not different from the control group. Forty two days post AI, cows treated on day 0 (10.74±2.68 ng/ml) and those treated at day 7 post AI (15.25±2.41 ng/ml) both had higher LSMeans for milk P_4 concentrations compared to the control group (2.83±2.99 ng/ml). Cows receiving hCG at 14 days post AI (9.41±3.01 ng/ml) had P_4 concentrations not different from those of cows in the other treatment groups.

Table 4. Effect Of Human Chorionic Gonadotropin Treatment Given Either At The Time Of Breeding, Day 7 or 14 Post Breeding, On Milk Progesterone Concentrations[*] In Holstein Dairy Cows.

Days Post Breeding	Treatment Group[a,b]			
	Day 0	Day 7	Day 14	Control
7	5.26±2.32	10.66±1.87	5.94±1.87	5.84±1.20
9	8.89±1.89	10.87±1.50	18.98±5.04	
11	11.39±1.70	11.26±1.50	26.31±5.04	
14	9.80±2.12	11.94±1.87	10.23±1.87	7.93±1.98
16	11.60±1.91	13.24±1.62	24.65±3.19	
18	12.00±1.19	13.52±1.50	28.78±3.19	
21	9.92±2.68[ab]	12.60±2.03[a]	10.41±2.03[ab]	7.19±1.98[b]
28	15.97±2.68[b]	12.53±2.21[b]	13.17±2.42[b]	4.94±2.38[a]
35	12.68±3.02[ab]	14.86±2.41[ab]	17.76±2.68[a]	8.72±2.99[b]
42	10.75±2.68[a]	15.25±2.41[a]	9.14±3.01[ab]	2.83±2.99[b]

[a,b]Numbers with different letter superscripts within each row differ at the 5.00 % level of confidence (P<0.05.).

[*] Values are presented as least squares means for milk P_4.

Figure 3.   Effect Of Human Chorionic Gonadotropin
On Milk P4 Concentrations In Dairy Cattle (UBC)

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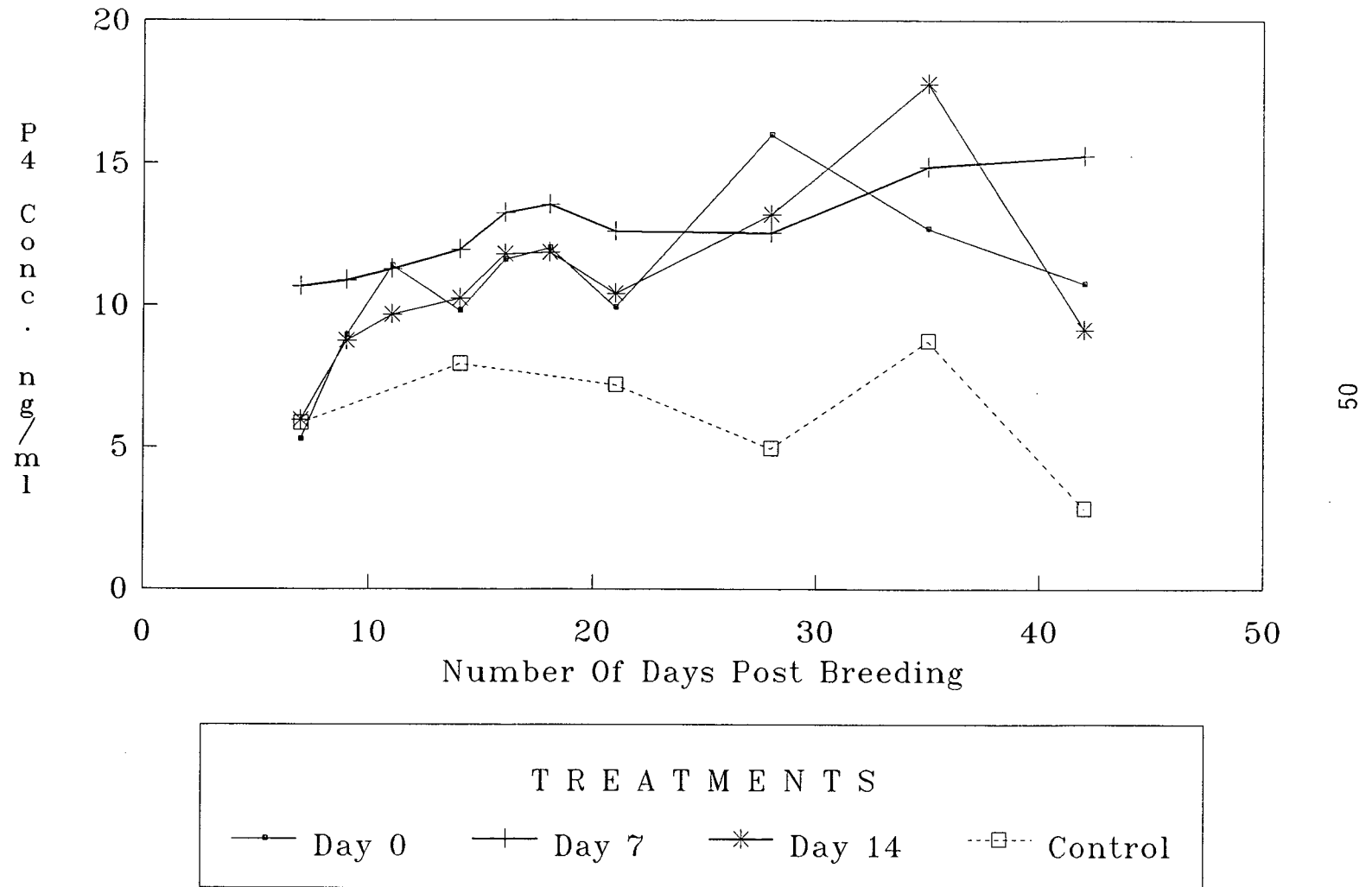
5.3.3. Effect Of hCG On Fertility

Three parameters were be used to evaluate the effect of hCG on fertility, viz; pregnancy rates, oestrous cycle length in non-fertile inseminations and capability to show oestrus in cows showing low P_4. Treatment with HCG on day 0 did not seem to extend the cycle length in the cow. Examination of P_4 profiles of cows that did not conceive shows that P_4 concentrations were better at evaluating the effect of hCG on cycle length compared to reports on when individual cows were actually observed in oestrus. Four cows treated with HCG on day 0 had low P_4 levels at one time or another between days 14 and 42 post AI. One animal had low (0.02 ng/ml) P_4 both in milk and plasma by day 14 post AI. The P_4 profile of this cow was in agreement with the data on the growth and regression of the CL which was confirmed to have regressed at the ultrasound scanning conducted on day 14 post AI. The animal continued cycling every 21 days as determined by its subsequent return to both low P_4 and regression of the CL which were all detected on day 35 post the first oestrus. A second cow had low P_4 values on day 18 and was subsequently observed in oestrus on day 21. In a third case, the cow had low P_4 on day 21 but oestrus was not detected until day 42 post AI. The fourth and last of the inseminated but non-pregnant cows in this group had low P_4 on day 28 but only observed in oestrus on day 35 post AI.

Three cows receiving hCG on day 7 post AI returned to oestrus. While two of them had low P_4 concentrations by day 18 and detected in heat on day 21, the third cow only showed a decline in P_4 concentrations on day 28 post AI. This last cow was observed in oestrus on day 42 post AI.

Of the four cows not conceiving in the day 14 treatment group, three of them had low P_4 concentrations by day 21 post AI. These were only observed in oestrus on either day 21, 35 and 42 post AI. The fourth cow showed low P_4 concentrations and was observed to be in oestrus on day 35 post AI.

Five cows in the control group were observed to have low P_4 concentrations at one time or another between days 21 and 42 post AI. Ultrasound evaluation at day 7 post AI revealed that one of these five cows was actually cystic. The remaining four cows were found to have low P_4 concentrations either on days 21, 28, 35 and 42 post AI. While the cow that was observed to be in oestrus on day 21 post AI was bred later that day, the other three went undetected and were only reported to have been bred on day 42 after the first AI. It was noteworthy that more pregnancies were established among cows receiving hCG. The respective pregnancy rates for day 0, 7, or 14 hCG treated and control cows were 4/8, 7/9, 4/9 and 3/8, respectively.

This experiment established that the dominant follicle present during the luteal phase in the cow, is capable of being induced to ovulate and proceeds to form a structure not different from the spontaneous CL. This set a premise for its use as a method for supplementing endogenous P_4 production. The experiment also set a premise for an beneficial effect of hCG on pregnancy rates.

6. Experiment Two : Effect Of hCG Administration At Various Times Following Breeding On Progesterone Profiles And Pregnancy Rates In Dairy Cattle.

To evaluate the effect of hCG treatments on P_4 profiles and pregnancy rates, two field stations collaborated in the implementation of this experiment. These were a research farm in the Fraser Valley (farm # 1) and a research farm on Vancouver Island (farm # 2), both stations are within British Columbia.

6.1. MATERIALS AND METHODS: Farm # 1

6.1.1. Animals : General Management Practices

All cows presented for AI starting from and including August, 1989 were included in the trial. The trial was concluded in February, 1990. The cows utilized in the experiment were part of the 230 Holstein cows constituting the herd on the farm. During the time this experiment was conducted, average milk production record is 8403 kg with 3.02 % butter fat and 2.72 % protein. Cows were normally allowed 77 days of a dry period prior to parturition. The calving interval in this herd was 12.7 months.

The experiment commenced in August, 1989. This month was part of a period (April to October) during the summer when cows were maintained on pasture. This pasture consisted of a mixture of clovers, orchard grass and rye. During this period, the cows were also provided with grain for both maintenance and production. The criteria used in deriving the production ration was on a 1 part of grain to each 4 kg of milk produced. Five kg of this portion of the feed was presented to the animal in the milking parlour, split in

equal portions given to the animal at each milking time. The balance was given to each animal in the barn.

During the winter months (November to March) cows are mostly fed a diet consisting of grass silage and corn silage. These were provided to the animal at a ratio of 1:2, in that order. In addition, the cows were allowed free access to local hay. This hay was made from the clovers and grasses from the pasture mentioned above. The cows were also allocated with their full amount of grain feed as outlined above. In all, the ration was formulated to provide the cows with a 18 % protein in the diet.

It is noteworthy that all cows on the farm received neither copper nor selenium supplement in their diet. It has been common practice on the farm to provide selenium to these cows as intramuscular injections given some 3 months following calving.

The farm is part of a private herd health programme through which a veterinarian from the programme pays regular visits to the farm. This was undertaken every 3 weeks. Cows in the herd were normally examined by the veterinarian shortly after parturition, and any that were found to be lagging in accomplishing uterine involution were given a tetracycline uterine infusion at approximately 30 days post partum. Most cows were reported to have exhibited a "false" oestrus approximately 5 days following parturition. This was ignored and any subsequent demonstration of the same phenomenon was recorded. All cows were subject to certification by the veterinarian as being either fit for AI or requiring further veterinarian attention. Cows thus declared to be ready for AI were bred by day 60 post partum. Oestrus detection was normally done by the herdsmen early in the morning (05:00 to 06:00

hours) and prior to and at the afternoon milking (15:00 to 16:00 hours). Heat checks, each lasting 5 to 15 minutes, were conducted during the course of the day. Observation for oestrus were as indicated under materials and methods of experiment one.

All cows were bred using AI. An effort was made to try and ensure the cows were bred by AI approximately 12 hrs following a reported standing oestrus. Enforcement of strict abidance to this rule was, unfortunately, compromised. This was due to the fact that the farm was dependent on the services of technicians based in Chilliwack, a short drive away from the station. Despite the rather short proximity to the research station, the technician in-charge of the AI programme also catered for other farms in the valley. At the earliest, the technician only arrived to render his services at 10:30 hours each morning and at 15:00 hours in the afternoon. This was, however, standard practice for most commercial dairy farms served by the same technicians.

6.1.2. Treatments

Seventy-nine lactating dairy cows were randomly assigned to receive treatment with hCG in one of four treatment groups. A single intramuscular injection of hCG 1000 IU (1 ml of APL^R) was administered either at the time of AI (Oestrus=D0, n=20), day 7 (n=20), 14 (n=20) post AI, or no hCG treatment given (control, n=19).

6.1.3. Milk Sample Collection

Milk sample collection was carried out on days 0, 7, 9, 11, 14, 16, 18, 21, 28, 35 and 42 post AI or until the first observed

oestrus. All samples were stored at 4^0C and transported to the laboratories at UBC. Quantification of P_4 was carried out using a Coat-a-Count^R solid phase RIA kit described earlier.

6.1.4. Pregnancy Diagnosis

All cows not returning to oestrus prior to day 60 were examined to verify their pregnancy status. Pregnancy diagnosis was conducted at day 60 using the rectal palpation technique performed by a veterinarian from the herd health programme.

6.1.5. Statistical Analysis

The statistical model used and the effects included in the model are given earlier. Due to variability in the frequency of sampling, each data set was analyzed in two batches (sampling days 0-18 and days 21-42). Contingency tables in Chi-square analysis were used to test for treatment effect on pregnancy rates. Unless stated otherwise, all comparisons were made at the 5 % level of significance ($P<0.05$).

6.1.6. RESULTS : Farm # 1

6.1.7. Milk Progesterone Profiles

The profiles, LSMeans and analysis of variance on milk P_4 concentrations are presented in figure 4, table 5 and appendix 4, respectively. Although treatment with hCG did not increase milk P_4 profiles, there was a trend for higher profiles among cows receiving the treatment. The respective overall LSMeans for days 0 to 18 were 6.53±0.57, 9.80±0.44, 7.57±0.56 and 6.70±0.51 ng/ml for cows treated on day 0, 7, 14 or those receiving none. LSMeans for

milk P_4 concentration, however, tended to be higher in cows receiving hCG at various times following AI. Despite having higher profiles amongst cows receiving hCG, there were no detectable differences until day 16 post AI. LSMeans for milk P_4 concentrations were significantly higher in cows given hCG treatment on day 7 post AI (17.42±1.15 ng/ml) compared to cows treated either on day 0 (7.99±1.40 ng/ml), 14 days post AI (11.19±1.33 ng/ml) and the control group (9.18±1.36 ng/ml).

By day 18, cows treated on day 7 (12.76±1.20 ng/ml) had LSMeans not different from those of cows receiving treatment on day 14 (10.71±1.27 ng/ml). The day 0 treated cows, whose LSMeans for milk P_4 (8.65±1.59 ng/ml) were not different from those of cows treated on day 14, were also not different from those of cows not receiving any injections with hCG (6.35±1.62 ng/ml). Cows treated on day 7 and those treated on day 14 both had LSMeans for milk P_4 concentrations greater than those of the control group.

Table 5.   Effect Of Human Chorionic Gonadotropin Treatment Given
          Either At The Time Of Breeding, Day 7 or 14 Post
          Breeding, On Milk Progesterone Concentrations* In
          Holstein Dairy Cows.

Days Post Breeding	Treatment Group[a,b,c]			
	Day 0	Day 7	Day 14	Control
0	0.78±1.40	0.76±1.25	-0.15±1.37	0.79±1.29
7	5.37±1.60	5.93±1.11	5.10±1.40	4.42±1.29
9	6.84±1.50	9.55±1.11	7.07±1.34	7.46±1.29
11	8.11±1.50	11.32±1.11	9.24±1.34	8.98±1.23
14	7.93±1.40	10.90±1.15	9.82±1.27	9.70±1.23
16	7.99±1.40[a]	17.42±1.15[b]	11.19±1.33[ab]	9.18±1.36[a]
18	8.65±1.59[a]	12.76±1.20[b]	10.71±1.27[ab]	6.35±1.62[ab]
21	6.29±2.08	10.51±1.44	9.47±1.74	9.12±1.99
28	8.46±3.78	6.63±2.02	6.12±2.13	3.22±2.31
35	3.43±3.83	8.49±2.47	9.82±3.15	6.83±2.80
42	3.37±3.83	9.04±2.92	8.17±2.82	4.75±3.16

[a,b,c]Numbers with different letter superscripts within each row
are different at least at the 5.00 percent level of
confidence (p<0.05).

*   Values are presented as least squares means for milk P_4.

Figure 4. Effect Of Human Chorionic Gonadotropin
On Milk P4 Concentrations In Dairy Cattle (Farm # 1)

TREATMENTS

- Day 0   + Day 7   * Day 14   Control
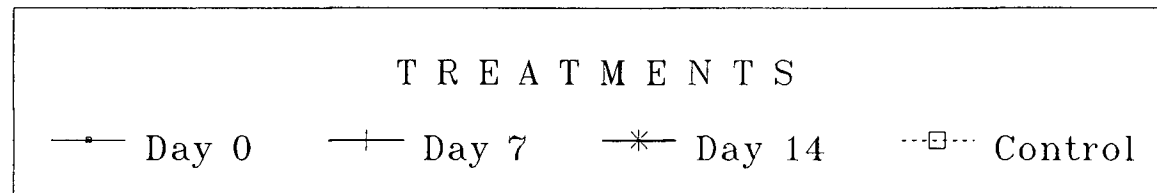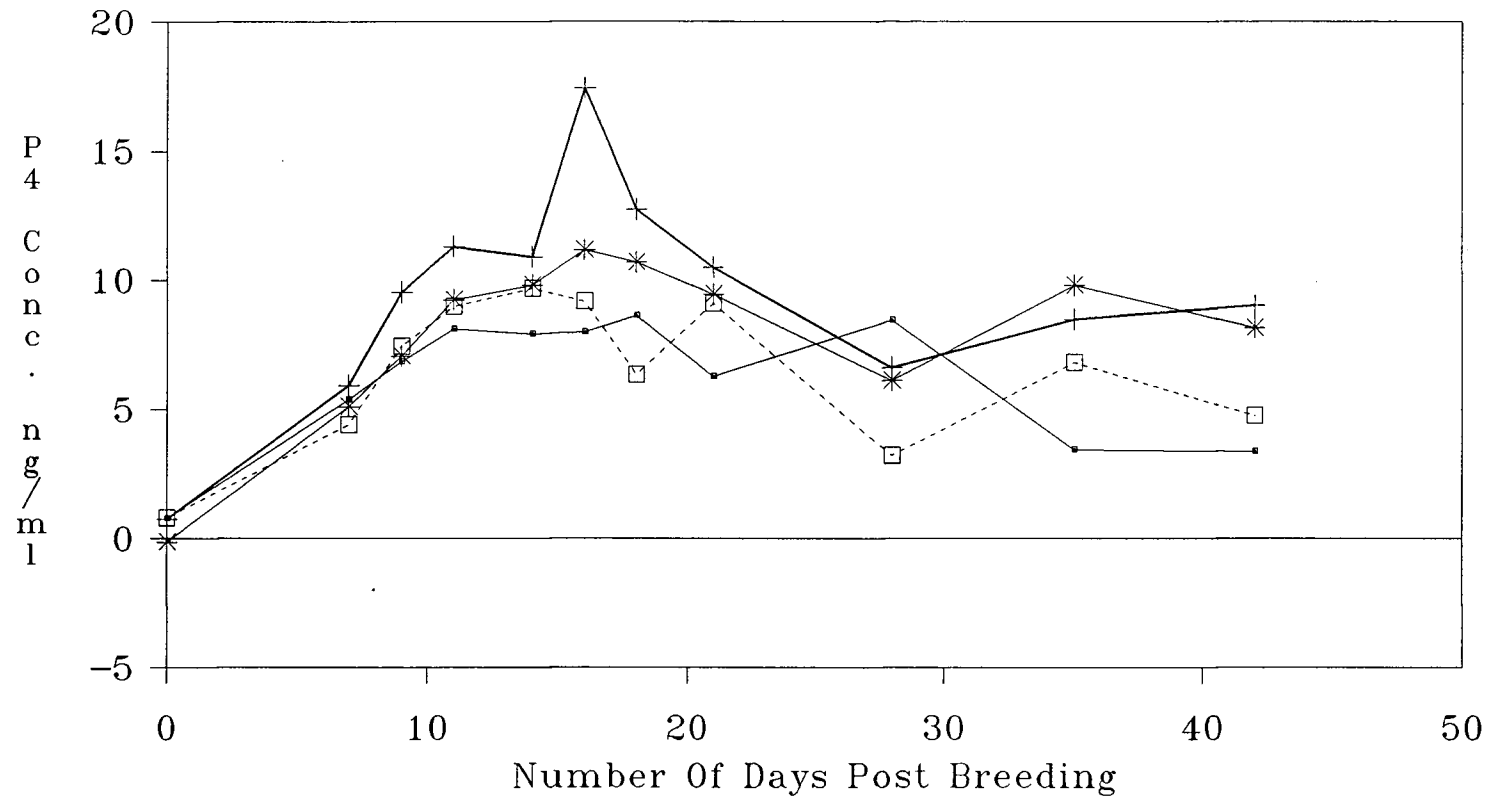
6.1.8. Effect Of hCG On Pregnancy Rates

Treatment with hCG lead to significant (P<0.01) increase in pregnancy rates (table 6). Pregnancy diagnosis at day 60 conducted by palpation per rectum showed that out of 20 cows treated in each of the day 0, 7 and 14 treatment groups, 25.00, 35.00, and 35.00 percent, in that order, were diagnosed pregnant. There was a 21.05 percent pregnancy rate among 19 cows constituting the control group.

Table 6.   Effect of Human Chorionic Gonadotropin Treatment Given Either At The Time Of Breeding, Day 7 Or 14 Post Breeding, On Pregnancy Rates[*] In Holstein Dairy Cows.

Site	Treatment	Day 0	Day 7	Day 14	Control	Total
farm # 1		5/20	7/20	7/20	4/19	79
(%)		(25)	(35)	(35)	(21)	
farm # 2		9/29	16/32	12/29	8/31	121
(%)		(31)	(50)	(41)	(26)	
Grand Total		14/49	23/52	19/49	12/50	200
(%)		(29)	(44)	(39)	(24)	

[*]   hCG significantly increased (P<0.01) pregnancy rates in treatments given on either day 7 or 14 post breeding.

6.1.9. Effect Of hCG On Cycle Length and Exhibition Of Oestrus

Levels of P_4 concentrations were used to determine the time when cows returned to oestrus. Based on P_4 profiles, 13 cows treated with hCG on day 0 were found to have low P_4 levels at various times ranging from 14 to 42 days post AI (figure 5) some of which were also observed to be in oestrus by day 42 post oestrus (figure 6). There was one case of low P_4 by day 14 post AI and these were subsequently low again on day 35 post AI. The cow was, however, not detected until discontinuation at day 42 post AI.

Four cows had low P_4 levels, two on days 16 and the other two on day 18 post AI. The remaining seven cows had low P_4 levels either on day 21 (n=5), 23 (n=1) or 28 (n=1). Based on the P_4 levels, it appeared that the cow with low P_4 levels on day 23 was cystic. One cow had high P_4 levels until day 42 post AI but was reportedly rebred on day 56 post AI. Four cows were rebred on day 21, one on day 24, one on day 26, one on day 30 and another on day 39 post AI. The remaining cows still had high P_4 levels on day 42 post AI. There is no data to indicate when these cows returned to oestrus.

Among cows treated on day 7 post AI, 14 were found to have low P_4 between day 21 and 42. Four were found to have low P_4 levels on day 21 post AI, 3 were rebred the same day. The other cows was rebred on day 28. Four more cows were reported to have been rebred by day 28. No data is currently available on the remainder of the cows. Twelve cows treated on day 14 post AI were found to have low P_4 concentrations at various times after AI. Out of these cows, 6 had low P_4 either on day 18 or 21 post AI. While four of these were rebred on schedule (day 21), the other two went undetected until a

subsequent oestrus on day 42 post AI when one of them was rebred. Two cows had low P_4 concentrations on day 24 and were observed to be in oestrus on the same day. Hence they were both rebred on day 24 post AI. Another two cows had low P_4 levels on day 28 post AI. One of them was rebred on day 30 while the other was rebred on day 50 post AI. One cow was found to have consistently low P_4 concentrations way into late dioestrus and was, therefore, assumed to have been cystic. No complete data for P_4 concentrations was available for the last cow.

Among the cows not receiving treatment with hCG, two had relatively short oestrous cycles. One had low P_4 concentrations on day 14 post AI and these subsequently rose and remained high until the last recorded sample on day 35 post AI. The other cow that had low P_4 concentrations on day 16 was rebred on day 18 post AI. Normally P_4 concentrations begin to fall starting on day 18 post AI and hit a nadir on day 21 (day of oestrus). Such was the case in five cows and all of them were rebred by day 21 post AI. One cow had low P_4 concentrations on day 24 and was subsequently rebred on that day. Two other cows were found to have low P_4 profiles on day 28 post AI. Both, however, did not show any signs of oestrus until day 53 when one is reported to have been rebred. No data is available for the other cow. On day 35, P_4 levels were low in one cow although the cow was reported to have been bred again on day 60. No information regarding its reproductive performance during the interim period was immediately available. The last cow had low P_4 levels on day 42 post AI but did not show any decline in P_4 values during the intervening period.

Figure 5.   Effect OF Human Chorionic Gonadotropin
On Oestrous Cycle Length In Dairy Cattle (Farm # 1)

Number Of Days To Low progestrone Level In Milk

■ Day 0   ▨ Day 7   ▧ Day 14   ▨ Control
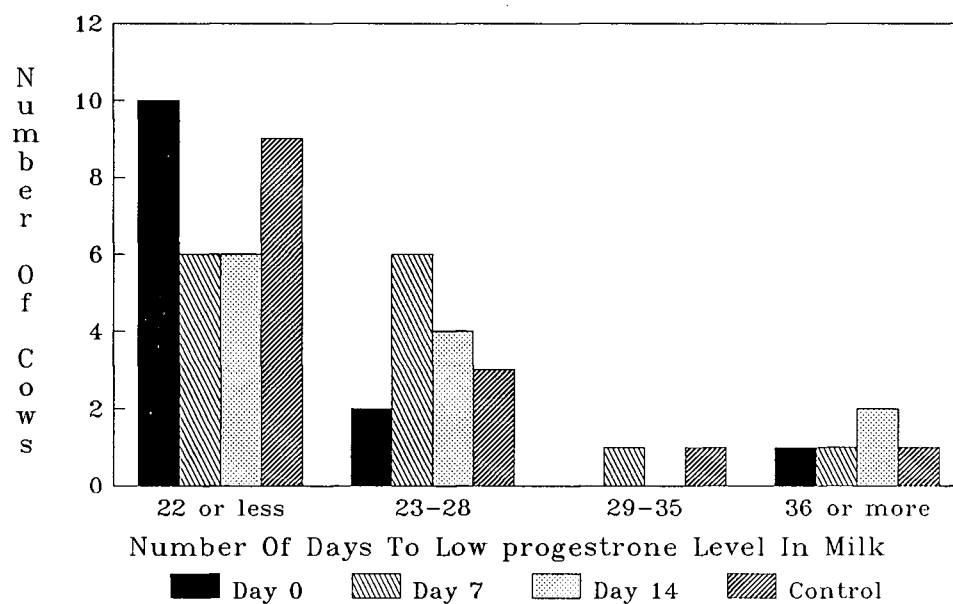
Figure 6.   Effect Of Human Chorionic Gonadotropin
On Exhibition Of Oestrus In Dairy Cattle (Farm # 1)

Number Of Days To Observed Oestrus

■ Day 0   ▨ Day 7   ▧ Day 14   ▨ Control
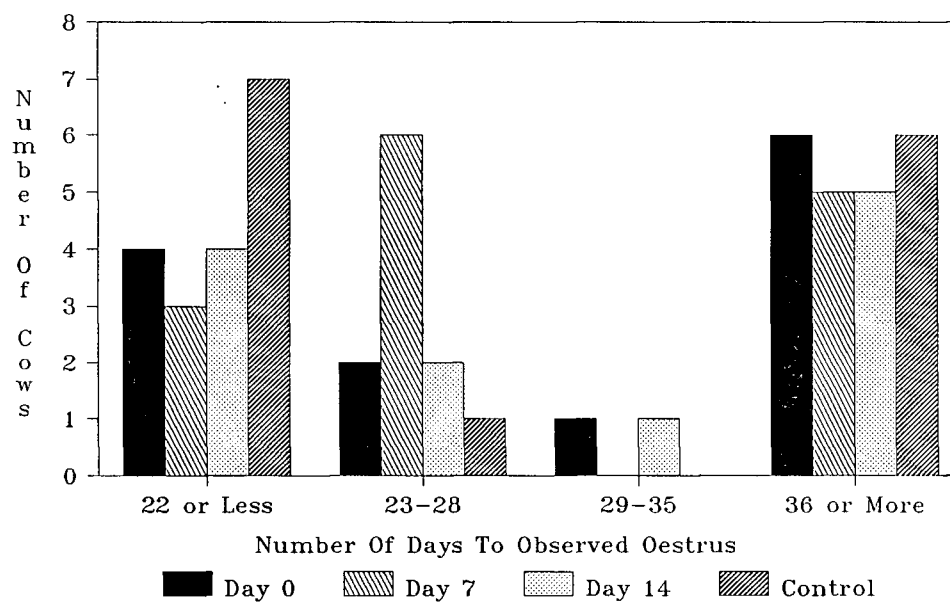
6.2. MATERIALS AND METHODS: Farm # 2

6.2.1. Animals: General Management Practices

The cows included in this trial were part of the dairy herd of Holstein cows on farm # 2. In total, there were 340 Holstein cows, 150 of which were lactating when this experiment was conducted. Twenty-five to 30 cows were in the dry herd. Cows included in the trial were those presented for AI between September 18, 1989 and January, 1990. Cows were milked twice daily at 03:00 and 14:00 in a herring bone milking parlour. These cows produced, on average, 8436 kg during the period this experiment was conducted. The average % fat and protein were 3.20 and 2.96, respectively.

During the first 2 months of the trial, the cows were on pasture and concentrates for their nutrition. The pasture consisted of orchard grass while the concentrate portion of the feed (16 % protein) was made up of 3 kg of barley mash and grain. Each animal was allocated 4 kg per day to meet its maintenance requirements. Extra grain, at a ratio of approximately 1 kg of grain to 4 kg of milk produced, was given to each animal in order to meet the requirements for production. This concentrate ration also contained mineral supplement but did not include copper, excluded due to a toxicity problem experienced by the farm. It was standard practice on the farm to give each animal an intramuscular injection of selenium at the time of drying off. Dry cows were not provided with any mineral supplements.

After October 1989, the cows were fed based on the above mentioned concentrate plus silage. The silage was made up of a 60 % corn to 40 % grass silage that together delivered 11.7 % protein. In addition, approximately 2 kg of hay (19.2 % protein) was given

to each cow. All feed was provided to the animal in the barn. Although the farm belongs to a herd health programme, it was not common practice to have cows examined prior to the first AI post partum. A veterinary evaluation was only resorted to if an animal failed to exhibit cyclicity well beyond 60 days following parturition. The first AI post partum normally took place around 60 days following calving, but could range from 45 to 85 days. Most cows bred during the trial period fell between the 45 to 60 day period. Rarely was an animal verified to have resumed cyclicity following parturition. Emphasis was mainly on observing for oestrus at around day 60 post partum.

There were no set times for oestrus detection but this exercise involved almost every cow hand available on the farm. This entailed that at most times during the day, there was at least one person checking cows for heat. Heat detection, as outlined under materials and methods of experiment 1, was mostly conducted at the time of collecting the animal in preparation for milking. This took place at 02:30 and 13:30 hrs every day. All AI were conducted by a trained farm technician. The timing of AI was based on observing for standing oestrus. Cows were bred 12 hrs following standing oestrus. Occasionally, timed inseminations, involving the use the luteolysin $PGF_{2\alpha}$, were used for problem cows failing to exhibit oestrus at expected times. AI was conducted 72 hrs following the administration of the luteolysin.

6.2.2. Treatments

One hundred and twenty-one lactating dairy cows were randomly assigned to receive treatment with hCG in one of four treatment groups. A single intramuscular injection of 1000 IU of hCG (1 ml of APL^R) was administered either on day 0 (n=29), day 7 (n=32), 14 (n=29) post AI, or no treatment (control, n=31). Although this many cows were involved in the study, milk samples were only collected from 23, 27, 24 and 26 cows receiving hCG either on day 0, 7, 14 post AI or not receiving any (control), in that order.

6.2.3. Milk Sample Collection

Weekly milk samples were collected starting on day 0 until day 42 post AI or until the first observed oestrus, whichever came first. All samples were stored at 4^{0}C and transported to the laboratories at UBC. Quantification of P_4 was carried out as described under materials and methods of experiment 1.

6.2.4. Pregnancy Diagnosis

All cows not returning to oestrus prior to day 60 were examined to verify their pregnancy status. Pregnancy diagnosis was conducted at day 60 using the rectal palpation technique by a veterinarian from the area herd health programme.

6.2.5. Statistical Analysis

Data for milk P_4 concentrations were analyzed using the procedures stated under experiment 1. Due to the homogeneity of the sampling periods (frequency), the data was analyzed in one batch.

6.2.6. RESULTS: Farm # 2

6.2.6.1. Milk Progesterone Profiles

The profiles, LSMeans and analysis of variance on P_4 levels are presented in figure 7, table 7 and appendix 5, respectively. Overall, hCG significantly increased (P<0.01) milk P_4 levels in cows receiving the treatment at either 7 (13.01±0.56 ng/ml) or 14 (10.65±0.58 ng/ml) days post AI. Cows receiving the treatment on day 0, however, had P_4 levels not different from those of the control group. Their milk P_4 levels were 7.54±0.74 and 8.39±0.73 ng/ml, respectively. P_4 Levels in milk also tended to increase with time.

Significant interactions between treatment and time made it difficult to ascribe the significant increase in P_4 to either treatment or merely time (sample number). Nonetheless, there were no differences between LSMeans for milk P_4 levels at oestrus when cows were randomly assigned to receive hCG at the respective time. This lack of divergence between means persisted until day 7 post AI. At 14 days post AI, cows receiving treatment at day 7 had significantly (P<0.05) higher P_4 levels (12.38±1.27 ng/ml) compared to cows that received treatment at oestrus (8.65±1.36 ng/ml). There were no detectable differences between cows treated either on day 14 (10.46±1.27 ng/ml) or those not receiving treatment with hCG (11.09±1.37 ng/ml) when compared to the day 7 treated cows. Comparisons made at day 21, however, show both day 7 (13.75±1.27 ng/ml) and day 14 (10.31±1.31 ng/ml) treated groups of cows as having significantly higher (P<0.01) levels of P_4 when compared to either control group cows (5.74±1.36 ng/ml) or those treated on day 0 (4.05±1.40 ng/ml).

At day 28 post AI, cows treated on either day 7 (14.22±1.44 ng/ml) or day 14 (13.59±1.47 ng/ml) both still had significantly higher (P<0.01) P4 levels than those treated on day 0 (6.29±1.94 ng/ml). Although these cows also had levels of P_4 tending to be higher than the control group (10.70±2.14 ng/ml), no significant differences, however, could be detected between their respective treatment LSMeans for milk P_4 levels. At day 35, milk P_4 remained elevated in cows treated on either day 7 or 14 (17.67±1.59 and 18.77±1.63 ng/ml, respectively) when compared to levels of the same hormone in cows either not receiving (9.79±2.27 ng/ml) or those administered with hCG on day 0 (9.55±2.04 ng/ml).

Forty-two days post AI, cows treated on day 7 (26.78±1.72 ng/ml) had significantly higher (P<0.01)1) P_4 levels than any other treatment group. Corresponding P_4 levels, in cows either not receiving hCG or those administered with the hormone on day 0 or at day 14, were 14.42±2.44, 17.34±2.46, and 15.92±1.63 ng/ml, in that order.

Table 7.   Effect Of Human Chorionic Gonadotropin Treatment Given
           Either At The Time Of Breeding, Day 7 or 14 Post
           Breeding, On Milk Progesterone Concentrations[*] In
           Holstein Dairy Cows.

Days Post Breeding	Treatment Group[a,b]			
	Day 0	Day 7	Day 14	Control
0	1.19 ± 1.74	-0.01 ± 1.53	0.68 ± 1.36	1.09 ± 1.52
7	5.72 ± 1.55	6.26 ± 1.27	4.79 ± 1.27	5.92 ± 1.42
14	8.65 ± 1.36^a	12.38 ± 1.27^b	10.46 ± 1.27^{ab}	11.09 ± 1.37^{ab}
21	4.05 ± 1.40^a	13.75 ± 1.27^b	10.31 ± 1.31^b	5.74 ± 1.37^a
28	6.29 ± 1.94^a	14.22 ± 1.44^b	13.59 ± 1.47^b	10.70 ± 2.14^{ab}
35	9.55 ± 2.04^a	17.67 ± 1.59^b	18.77 ± 1.62^b	9.79 ± 2.27^a
42	17.34 ± 2.46^a	26.78 ± 1.72^b	15.92 ± 1.63^a	14.42 ± 2.44^a

[a,b]Numbers with different letter superscripts within
      each row are different at least at the 5.00 percent level of
      confidence ($P<0.05$).

[*]   Values are presented as least squares means for milk P_4.

Figure 7. Effect Of Human Chorionic Gonadotropin
On Milk P4 Concentrations In Dairy Cattle (Farm # 2)

TREATMENTS

—■— Day 0   —+— Day 7   —*— Day 14   ---□--- Control

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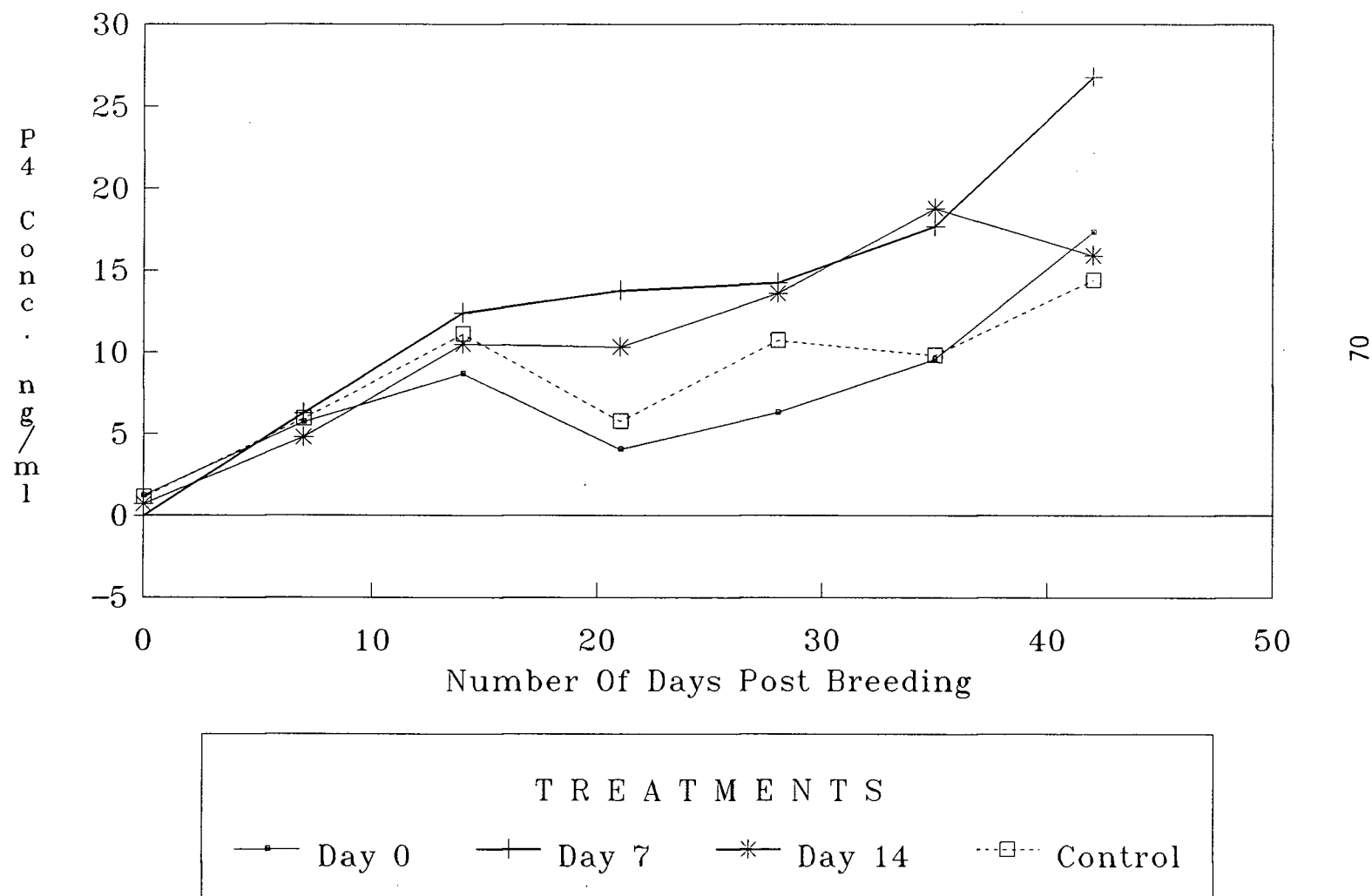
6.2.6.2. Effect Of hCG On Pregnancy Rates

Pregnancy rates were significantly improved by treatment with hCG given either on day 0, day 7 or 14 post AI (table 6). Out of twenty-nine cows receiving treatment on day 0, 31.00 % (n=9) were found to be pregnant using the rectal palpation technique undertaken at 60 days post AI. The respective percentage of cows conceiving to the inseminations done prior to treatment with hCG on either day 7 or 14 post AI were 50.00 (n=16) and 41.40 (n=12). The corresponding values for the control group were a 25.80 % (n=8).

6.2.6.3. Effect Of hCG On Cycle Length and Exhibition Of Oestrus

For effects of hCG on oestrous cycle length in non-fertile inseminations refer to figure 8 and 9. Nine cows were found to have low P_4 levels on day 21 post AI. One cow had low P_4 on day 24 post AI was observed in oestrus the same day. Two cows were found to have low P_4 profiles on day 14 post AI. Both were not detected until day 42 post AI. Two cases of cows that had low P_4 levels on day 35 and 38 were recorded. Only the latter was detected in oestrus on day 38 post AI.

Among cows receiving hCG on day 7 post AI, 9 cows had low P_4 levels prior to day 42 post AI. One cow had a short cycle whose duration was 14 days. It is not clear whether this cow returned to oestrus or not. Three cows had low P_4 levels by day 21 post AI. While one of them was rebred on day 21, the other two were rebred on day 24 post AI. One cow had low P_4 levels on day 26, another on day 28, each one of them being rebred on the day when they showed low P_4 levels. Two cows had high P_4 levels until 33 and 35 days post AI, the former being rebred on the day its P_4 levels were low the

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latter was rebred 40 days post AI. The last cow had high P_4 levels until sampling ceased at day 42 post AI.

Amongst cows treated on day 14 post AI, 10 cows showed low P_4 levels. P_4 profiles of two cows did not decline until day 42. There was one case of a short cycle lasting 14 days and was rebred on the same day. Five cows showed low P_4 levels on day 21 post AI. Three of these 5 cows, however, had high P_4 levels until day 42 post AI. While the fourth cow was rebred on day 24, the last one was rebred on day 38 post AI. Two cows were found to have low P_4 levels on days 25 and 26 post AI. Each one was rebred on the respective day when P_4 levels were low. The last four cows all had P_4 levels dropping to oestrus levels on day 28 post AI. Only one cow was rebred on the day coinciding with the low P_4 levels. The other three still had high P_4 levels at day 42 when sample collection was terminated.

P_4 levels were low in 15 cows in the control group. Eleven of these cows had low levels of P_4 on day 21 post AI. However only 6 were rebred by day 22 post AI. Two cows were rebred before day 30 post AI, one on day 25 and the other on day 28. The remaining 4 were not observed in oestrus until day 42 when sample collection ceased. Of the remaining 3 cows, one had low P_4 profiles on day 25 and another on day 28. Both were rebred on day 28 post AI. For the last cow, levels of P_4 were low on day 38, and was rebred on the same day.

Figure 8. Effect Of Human Chorionic Gonadotropin On Oestrous
Cycle Length In Dairy Cattle (Farm # 2).
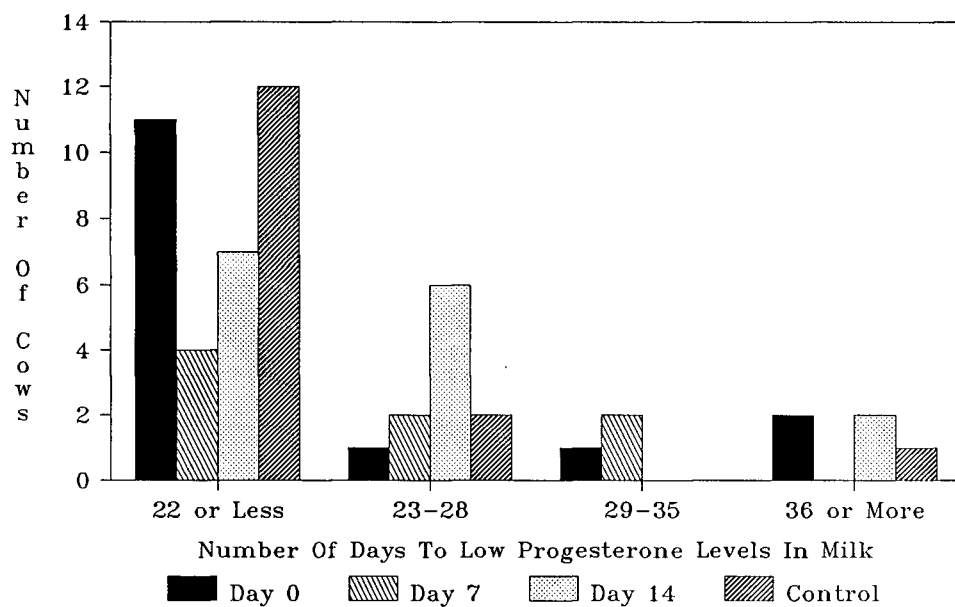
Figure 9.   Effect Of Human Chorionic Gonadotropin
On The Exhibition Of Oestrus In Dairy Cattle (Farm # 2).
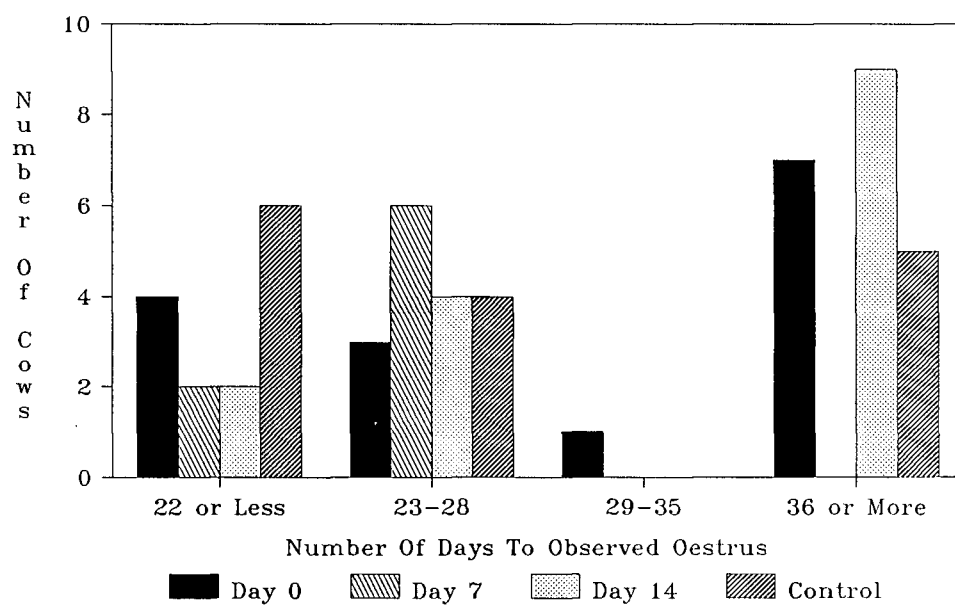
7. DISCUSSION

Techniques for reducing losses in reproductive efficiency in livestock have eluded mankind for time immemorial. Much ground has been covered in of elucidating, first what forces predispose the losses in reproduction and secondly finding ways and means for overcoming compromised fertility. Although we are far from understanding the agents causing the demise of embryos (Heap, 1985), it is becoming increasingly clear that some factors predisposing early embryonic mortality are susceptible to manipulation at one point or another. The data to under discussion is just one example. The purpose of conducting this experiment was to develop a viable method of supplementing endogenous P_4 concentrations. It was intended to emulate nature in exemplified by the mare wherein "new" CL are developed some 45 to 60 days post oestrus (Hafez, 1976). These accessory CL are developed when the spontaneous CL begins to exhibit diminished functional efficiency.

7.1. Induction Of Accessory Corpora Lutea

One of the aims of the first experiment was to determine if giving intramuscular injections of hCG, at various times following breeding, would induce the development of accessory CL. Treatment with hCG increased the number of CL in cows treated on day 0. Such a finding is corroborated by results obtained by Price and Webb, (1989). The report of these authors is the only one that documents visualizing, with the aid of a laparoscope, the effects of treatments such as hCG given during the luteal phase in the bovine. The increased number of ovulations observed in the group of animals treated on day 0 could be attributed to the effect of hCG for two

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reasons; firstly, the incidence of twin ovulations in cattle is low (Price and Webb, (1989), secondly the ultrasound scanning conducted on day 7 showed that in one cow, the appearance of one CL was not typical of that found at this stage of the oestrous cycle. The greatest potential to be realised from using hCG on day 0 lies in its capability to synchronize ovulations at oestrus and possibly increase the frequency or number of these ovulations (Wagner et al., 1973).

The present study shows that 7/9 of the cows treated on day 7 developed accessory CL. Similar results were reported by Price and Webb, (1989). These authors found that 83 % of heifers receiving hCG given during the early luteal phase (days 4 to 7 post oestrus) developed accessory CL. The ovulatory potential of the dominant follicle was the subject of a recent report by Savio et al., (1990). The dominant follicle was the ovulatory one in almost 93 % (n=40) of the heifers following treatment with a luteolysin (PGF$_{2\alpha}$ or PGF$_{2\alpha}$ analogue) given on day 7 post oestrus. Similarly, Walton et al., (1989) evaluated the ovaries of beef heifers treated with hCG on day 5.5 and slaughtered on day 12. The spontaneous CL was accompanied by a "new" 4-day old CL. However, only 50 % of the animals treated on day 14 responded by developing accessory CL as a consequence of the day 14 treatments with hCG. Similar results were reported by Price and Webb, (1989). In their experiment, 66 % of the animals receiving the hCG treatment between days 14-16 post oestrus developed accessory CL. McDermont et al., (1986) also found a higher frequency of accessory CL in animals given hCG on day 15 post oestrus.

de Los Santos-Valadez, (1982) gave hCG on day 15 post oestrus and found 25 % of the animals receiving hCG had palpable accessory CL. When animals were examined at caesarian section, Greve and Lehn-Jensen (1982) found that hCG given every other day starting from day 13 until day 35 had stimulated the ovaries, the number of accessory CL ranged from 2 to 5. Wiltbank et al., (1961) also found 1 to 3 accessory CL in 67 % of the pregnant heifers treated with daily injections of hCG starting on day 14 until day 34.

The fact that only 7/9 of the cows developed accessory CL indicates that a fair number of follicles failed to respond to the gonadotropin. The reduced response, and the variation from other values quoted in other experiments, could be due to a variety of reasons. Variations in accessory CL development could be found in postulations that in the cow and sheep (Webb et al., 1978; England and Webb, 1979; Webb and England, 1979) the binding of hCG to the follicular wall is very variable and the binding capability is only limited to one or two large antral follicle(s). Mertz et al., (1981) presented evidence, based on follicular fluid content of P_4 and/or oestradiol (E_2), indicating that the large day-6 follicles in the cow were less likely to be atretic. The above authors' observations were in agreement with postulations of Rajakoski (1960) and both the in-vitro and in-vivo studies of Bennett et al., (1989). The latter authors found greater response to hCG treatment when this was administered during the early luteal phase (days 3-7), a phase when receptors to LH/hCG were reported to be maximal. It is possible, too, that cows show a diminished response to such treatments (de Los Santos-Valadez et al., 1982) or that day 7 could have been premature to administered the treatments. Even though

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these follicles were growing, they probably still did not have the full complement of receptors (Lavoir and Fortune, 1990).

Still less animals developed accessory CL from treatments given at late to mid-dioestrus (day 14-15 in the present experiment). It is possible that most of the dominant follicles present during dioestrus might have been atretic or regressing (Rajakoski, 1960, Mertz et al., 1981). Evidence supplied by Ireland and Roche (1982, 83) indicates that once ovarian follicles become atretic, their capacity to bind LH/hCG diminishes dramatically. A reduction in the number of receptors during dioestrus has been documented (Ireland and Roche (1983), indicating that less receptors were available at the time we report the least response (day 14). Lavoir and Fortune (1990) have also recently reported that there appears to be congruence between the attainment of morphological dominance and functional dominance. The two phenomena coincide during the first wave of follicular growth (early plateau phase), this coincidence is lost as the follicles proceed in development (late plateau phase). Solely using ultrasound, it is not easy to detect these differences.

It is apparent from the foregoing that follicles do respond to ovulatory doses of gonadotropins. That responsiveness seems to be limited to a window such as the one identified in this experiment (day 7 post oestrus).

7.2. Growth of Corpora Lutea

The effect of hCG on the formation, growth and function of the CL was clearly demonstrated by comparisons between hCG-treated versus control animals. Treatment with hCG lead to significant

increase in CL diameter. This premise of the effect of hCG on the CL was sustained until day 42. Initial linear increase was evident and this was taken to be indicative of the response of spontaneous CL to the luteotropic effect of hCG. The growth of these induced CL was superimposed on the effect of hCG on the spontaneous CL. The CL which were induced by the treatment grew to an extent that at one point it was not possible to distinguish between spontaneous versus induced CL. Such a difficulty was also experienced by Price and Webb, (1989).

No data is presently available in the literature to warrant any comparisons on the formation and growth of the CL. Most data examined is only limited to citing the development of accessory CL but lack biometrical measurements of these structures. Results obtained from this study provide evidence that the induced CL are maintained for a considerable period of time during gestation. The only short coming of the experiment is that it did not follow the fate of these induced CL and their functional capability remains undetermined.

7.3. Progesterone Profiles

Elevated P_4 concentrations in female animals, have been shown to be indispensable for the establishment and maintenance of pregnancy (Hammond, 1927; Estergreen et al., 1968). It has been shown that such levels must be maintained at a higher than basal level (Staples and Hansel, 1961), failing which pregnancy is negated (Bulmann and Lamming, 1978). Levels of P_4 in an oestrous cycle preceding (Folman et al.,1973; Fonseca et al., 1983) or proceeding a particular insemination (Erb et al., 1976; Lukaszewska

and Hansel, 1980; Thomson et al., 1980) have been shown to be associated with prevailing levels of fertility.

Differences in plasma P_4 concentrations between hCG treated versus control cows in the current study were present only on days 18, 35 and 42 post breeding. Similar results were reported by McDermott et al., 1986, de Los Santos-Valadez et al., 1982) Interesting enough, results reported by de Los Santos-Valadez et al., (1982) indicate that this difference was only evident in heifers and not in cows. Responses such as this one lead to the supposition that animals may differ in their capability to respond to gonadotropins depending on their age status. Bennett et al., (1989) also reported significant differences in plasma P_4 concentrations starting at day 4 until day 8 post oestrus.

Conversely, Morris et al., (1987) suggested that there are no differences between P_4 concentrations among cows having single ovulations compared to those having twin ovulations. This assertion lead Price and Webb, (1989) to believe that collection of blood for P_4 concentration determination may not be worthwhile. It could be argued, however that these induced structures are functional. If such is the case, then it seems reasonable to assume that peripheral levels of P_4 should be different at some point in time. Whether or not this lack of divergence is due to a non-functional state of induced CL, is yet to be established. Results obtained from this study seem to indicate that these accessory CL are functional. This lack of agreement regarding differences between animals bearing twin versus those carrying only the spontaneous CL, may be attributed to the use of peripheral blood.

The earliest detectable differences in milk P_4 occurred on day 14 post breeding among animals on farm # 2. Similar differences were observed also in the animals at the South Campus of UBC, although differences in this case were only evident starting from day 21 post breeding and persisted until day 42 post breeding. The P_4 concentrations of the animals on farm # 1 were different between hCG-treated versus control cows only on days 16-18 post breeding.

The present experiment detected a tendency towards lower milk P_4 concentrations among animals receiving hCG on day 0. Such a finding is in agreement with results reported by Echternkamp and Maurer (1983). There is no immediate explanation for this inconsistency in the variations of P_4 profiles in these groups. It is improbable that the failure to establish pregnancy in a majority of animals on farm # 1 might have contributed to lowering the effect of hCG on P_4 concentrations (Lewis et al., 1990). There are speculations, however, to the effect that different animal populations and, indeed, different batches of the hormone administered will in some way influence the response by animals receiving the treatment (de Los Santos-Valadez et al., 1982).

It is possible that differences in P_4 concentrations could have been significant at the utero-ovarian vein. Such a conclusion is supported by the work of Ireland and Roche (1983) who failed to detect any changes in serum P_4 concentrations despite showing several fold increases in follicular fluid content of P_4. This fact merely underscores the suggestion that hormones in peripheral circulation are greatly diluted. The lack of significant increases in P_4 concentrations among cows treated on day 0 may have been due to the reduction in the size and number of small and large luteal

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cells, respectively (sheep, Alila and Hansel, 1984; pig, Wiesak, 1989). Such treatments with hCG given in late-dioestrus, were reported to induce morphological changes of the luteal cells. The reduction in the small luteal cells could have contributed to the lack of significant increases in P_4 concentrations reported in the present study. This was, however, in contrast with the results reported by Bennett et al., (1989) who found no such morphological changes when animals received hCG later in the oestrous cycle.

It is possible the lack of significant increases in P_4 concentrations in animals receiving hCG might been mediated via reductions in LH receptors seen between days 10 and 14 post oestrus (Mee et al., 1990). These results were done using CL preparations from animals receiving hCG either at oestrus or 2 days post oestrus. Similar suggestions were proposed by Lucy and Stevenson (1986).

It should be appreciated, however, that P_4 concentrations in animals tend to be very variable. This variability has been attributed to differences in development rates of CL and the oscillatory nature of P_4 secretion during the luteal phase (Stubbings and Walton, 1986).

7.4. Pregnancy Rates

Estimating the financial costs that are imposed by losses in pregnancy is a very difficult task to accomplish, amounting to US $1.40 million per annum in the US (Roberts et al., 1990). Flint et al., (1990) on the other hand, estimated such losses as being in excess of £300 million sterling per annum for the dairy and beef industry in the United Kingdom.

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The use of hCG in the current experiment significantly increased pregnancy rates, more so when given on day 7 or 14. Such a finding is corroborated by some reports (Hansel et al., 1976; Echternkamp and Maurer, 1983 in heifers; Babbler and Hoffman, 1974; Wagner et al., 1973; de Los Santos-Valadez et al., 1982; McDermott et al., 1986 in animals with compromised fertility). Reports of no significant effect of the treatment are also documented (Hansel et al., 1976 in beef cattle; Looney et al, 1984). Conversely, evidence of a negative effect on pregnancy has also been advanced (Hansel et al., 1960 in dairy cattle; Ecternkamp and Maurer, 1983 in cows; McDermott et al., 1986 in cattle of high fertility).

Although the pregnancy rates in the control group of the UBC South Campus herd was comparable with those obtained in an earlier study (Rajamahendran et al., 1989), corresponding rates for the two farms involved in experiment two were very low. Providing a concise breakdown of the reasons for such low rate of fertility is an insamountable task. Some of the losses in fertility could be attributed to wrong timing of inseminations, more so on farm # 1 where 23 % of the inseminations were carried out when P_4 concentrations were above a 1.00 ng/ml level and were either 3-5 hrs premature or overdue. These observations are incompatible with those observed in the trial conducted on farm # 2 where only 7 % of the inseminations were carried out when P_4 concentrations were still above the 1.00 ng/ml level.

Previous reports indicate that this is a problem fairly common to many a dairyman. Appleward and Cooke, (1976) reported that based on plasma P_4 concentrations, 10 to 20 % of the animals presented for insemination were not ready for insemination. On the other

hand, Hoffmann et al., (1976) based their study on milk P_4 concentrations and found that between 14 and 26 % of the animals were not in oestrus at the time they were bred. It was, however, reported by Foote et al., (1979) that 19 % of animals do show oestrus even though P_4 concentrations in milk are high. It is not clear as to how many of these are in true oestrus. We note from this study that on farm # 1, 3/18 of the animals inseminated when P_4 concentrations were high conceived.

Animals inseminated when ovulation has already occurred have been reported to exhibit compromised fertility (Trimberger and Davis, 1943; Boyd, 1970; de Kruif, 1978). One reason for this depression in fertility is that the ova are over aged or old (Lodge, 1976) by the time the animals are presented for AI. This has been associated with impairment in the development of the early embryo. An optimal time for conducting AI has since been defined (Hafs and Boyd, 1973). This was simplified by these authors to be; "....that all animals observed in oestrus in the morning should be bred that afternoon, while those observed in the evening to be standing to be mounted by other cows should be bred the following morning...."

It is plausible that some of the losses in pregnancy rates on farm # 2 could have been due to the first inseminations being done too early relative to parturition. It could be possible, too, that the failure to ensure that an animal was observed to have resumed cyclicity prior to breeding could have increased the incidence of animals failing to conceive. This is drawn from the fact that animals seen in oestrus by day 60 were bred irrespective of whether these cows were detected in heat since parturition. The first heat

exhibited following parturition is usually followed by a luteal phase of diminished duration.

The meagre pregnancy rates recorded in the control groups of the second experiment are only comparable to those obtained by (Monty and Wolf, 1974). While the adverse environmental conditions was one possible reason advanced in an attempt to explain such low rates of fertility, it is probable that the change in the feeding systems undertaken when the current experiment was underway may have played a part in influencing these fertility rates. The change involved a switch from an open grazing system based on a pasture consisting of mixture of grasses (timothy and rye) and clovers (red) to a winter schedule of individual feeding system.

Inherent in this change in the nutritional quality of the feed that the animals received was a possible change in the protein content of the feed. Rattray, (1977) reported that excess dietary protein was associated with increases in the basal rate of loss in embryos. Since nutrients required for reproduction are generally the same nutrients required for growth and milk production (Hafs and Boyd, 1973), it is not easy to accept this as an explanation for the reduction in fertility. No report is available indicting that animals in the study experienced loss in milk production. Conversely, indications are that production remained constant over the years.

The denial of elements such as selenium and copper to the animals on farm # 1, may have compromised fertility. The use of selenium to improve fertility rates in cattle is still unconvincing. Its major benefit is the reduction in the incidence of retained placentae (Ammerman and Miller, 1975; Trinder and

Renton, 1973) or development of a muscular condition (white muscle disease) among calves borne of cows deficient in selenium (Kincaid and Hodgson (1989), more so when the deficiency is severe (Weiss et al., (1990). Less severe cases cause metritis (Harrison et al., 1984). Deprivation of such other elements as copper, vitamin A and Iodine, have also been implicated in the incidence of retained placenta in cattle (Eger et al., 1985). Information available at this moment indicate no change in the rate of retained placentae or, indeed, white muscle disease. On the contrary, in a herd of 230 on farm # 1, only 2-3 cases are normally experienced each year. It should be realised, however, that the metabolism of these minerals in-vivo, is greatly influenced by how other elements are metabolised and vice versa. It might still be possible that the effect of inadequate selenium and/or copper could have been effected via changes in another element.

Wagner et al., (1973) suggested that the main benefit of administering hCG at the time or just prior to breeding mainly lies in its ability to influence the timing rather than increasing ovulation rates in treated animals. Results from this study and information available in the literature seems to support this view. Greater potential for increasing pregnancy rates in cattle seems to be the use of hCG on day 7 post oestrus. The response in this group of animals clearly shows superiority not only in developing accessory CL but also their capacity to secrete P_4. It is, no doubt, due to this that we also experienced the greatest increase in pregnancy rates among the same group of animals. The 24 % increase is the greater than any sited in the literature. Premise for this response was provided by the detailed study (experiment

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one) conducted at the South Campus of UBC where we found a 40 % increase in animals treated on day 7 post oestrus compared to the control group.

Hence, the contention that the use of hCG may only be beneficial in cases of established infertility (Diskin and Sreenan, 1985). This should be approached with caution, because the current study has established the appropriate time for giving such treatments and the discussion on P_4 profiles/cycle length have shown the adverse effects of rendering these treatments at certain times relative to oestrus. It must be resolved, however, what the dosage of hCG and the frequency of administering the hormone should be. It is not clear whether the route of administering the drug play any role in manipulating the responsiveness of animals. There seems to be much promise in realising better responses with use of hCG to improve fertility if only we can get evaluate contribution of each one of these factors that are so variable right now.

Diskin and Sreenan (1985) emphasized that the use of hCG to improve fertility, was not likely to produce conclusive results until we attain a better understanding of the endocrine processes involved in luteolysis and the establishment of pregnancy. The same problem can be adequately answered by obtaining a thorough understanding of follicular dynamics in the cow. Such again seems to be the advantage of the present study. The question, however, as to whether the follicles are present during the luteal phase in order to ensure optimal function of the CL, is yet to be answered. It might just be that the follicles are present in order to ensure luteolysis in the event of a pregnancy failing to establish.

This study has also identified the fact that pregnancy rates can be improved upon through treatments with hCG given on day 7 post oestrus. Whether the results obtained from this study can not be obtained if the animals with a high level of fertility were used, is another question that remains unanswered.

7.5. Cycle Length And Expression Of Oestrus

Treatment with hCG has received variable results in several aspects of reproductive physiology in the cow. Notable among these, and equally unresolved, is the effect of hCG on the length of the oestrous cycle in non-pregnant animals. Included for discussion, is the effect of hCG on the psychic expression of oestrus. Silent oestrus is one of the scapegoats that most dairymen use in an attempt to explain the high incidence of cows returning for service. It has, however, been stated that several cows in the herd do show signs of oestrus but due to inadequate observations, these go unnoticed (de Kruif, 1978). Animals observed continuously approached a 100 % rate of being detected in standing oestrus (Williamson et al., 1972). Casual observation, on the other hand, were only capable of detecting 50 % of cows in oestrus. Such observations lead one to conclude that most of what we have termed as "silent oestrus" may really be a reflection of the inadequacy reproductive management rather than an indication of physiological failure on the part of the cow to express oestrus. It would be interesting to find out through continuous observation of animals prone to exhibiting silent oestrus, if indeed the phenomenon exists at proportions we have ascribed to it. Several animals included in the two experiments showed low P_4 concentrations at one time or

another between days 21 and 42 post oestrus. For most of these animals, at least one cycle was not detected. The proportion of hCG-treated cows with low peripheral P_4 and detected in oestrus never exceeded 50 %, which was relatively lower than the 78 % detection rate for control cows.

This trend towards an increase in the number of cows not returning for service by day 21 was similar to rates reported by Macmillan et al., (1986) who used a GnRH agonist (buserelin). They noted a slight increase in the number of animals not returning to oestrus at day 21 post oestrus.

A slightly longer (8-10 day) extension of the cycle was experienced by cows treated on day 14. Similar results have been documented (de Los Santos-Valadez et al., 1982; Macmillan et al., 1986; Morris et al., 1976; Wiltbank et al., 1961; Eduvie and Seguin 1982; Seguin et al., 1977). Conversely, Helmer and Britt, (1986) who found no difference in days to first expressed oestrus following hCG treatment. This extension ranged from 2 to 8 days.

Indications from the present experiment are that the functional capacity of the CL of both the treated and control cows ceased at approximately the same time. There was, however, an impairment in the expression of oestrus as shown by the low number of animals actually observed in oestrus. This trend leads to contentions that hCG increased cycle length. Clearly, the P_4 concentrations corroborated the data on the diameter of the CL which showed regression of the CL at times corresponding to a decline in P_4 profiles. The functionality of CL cease on schedule and yet for some reason standing oestrus is suppressed, this points to an area that does not seem to have adequate answers available to

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date. The mechanism by which cows fail to exhibit oestrus call for further research in this area. The suppression of oestrus after day 21 merely allows a predicament to surface. Two factors have become confounded. Based on P_4 profiles, it was not possible to determine whether early embryonic mortality occurred in animals showing extended cycles. It could be that the extension in the length cycle was due to the hCG treatment.

Treatment with P_4, which result in higher P_4 concentrations, increases the rate of atresia in bovine ovarian follicles (Maracek et al., 1977). It should be borne in mind that the concentrations of P_4 at the ovarian vein/artery level are much higher (Ireland and Roche, 1983). This is a viable cause of atresia in these follicles. This suggested mode of action for P_4 does not agree with earlier reports indicating that P_4 treatments prior to oestrus increased the rate of detecting animals in heat (Stevenson et al., 1989). If such an effect was detected, it is reasonable to assume that the follicles present were oestrogen active, hence, non-atretic. There exists a possibility, however, that the timing and amount of P_4 administered may be the limiting factor in determining when atresia will set in.

The increase in P_4, although not that significantly improved at the peripheral level, may have altered the $P_4:E_2$ ratio (Roberson et al., 1989). This ratio of $P_4:E_2$ represents the balance at which the two steroids either in concert or individually, act to modulate pituitary function (Stumpf et al., 1988). P_4 alone may be the negative feedback on the pituitary and this may explain the increase in LH concentrations in animals receiving sub-normal levels of P_4 in the experiments of Roberson et al., (1989). These

increases in the gonadotropin may have merely been due to the removal of an alternative negative feedback mechanism originating from the ovary, possibly alterations in the steroid balance due to luteinization of follicles. This increase in gonadotropin levels (LH) most likely may have been accompanied by concurrent increases in inhibin (Padmanabhan et al., 1984). Increased levels of inhibin may modulate reproductive physiology by preventing the secretion of FSH from the anterior pituitary. This, which in turn, could have lead to failure in the development of receptors for LH. This mode of action is supported by results obtained by Padmanabhan et al., (1984). LH increased in concomitance with increases in follicular fluid content of inhibin. No collateral increases were reported for follicle stimulating hormone. This, further provides another possible causative agent for the process of luteinization. Hernandez-Ledezma et al., (1982) concluded that GnRH-induced LH release appeared to stimulate luteinization and possibly influenced the steroidogenic pathways of these follicles.

It is possible, too, that the change in the steroid balance in the follicles seems to be the failure of oestrogen-inactive (atretic) follicles to convert aromatize androgens. This lead to increases in the level of androgens in circulation, hence, indirectly increased the rate of atresia (Ireland and Roche, 1983). Cystic follicles exhibit extensive thickening of the thecal layer while granulosa cells may or may not be present (Hernandez-Ledezma et al., 1982). Due to these morphological alterations in cystic follicles, the content of follicular fluid varies f r o m   t h a t characteristic of normal follicles. This difference is due to the lack of granulosa cells which are indispensable for the

aromatization of androgens. Kesler et al., (1980) postulated that oestrogens produced by cystic follicles are sequestered within the follicle. Whether the luteinization of follicles observed during experiment one also precipitate such functional changes is not clear.

Farookhi, (1980) suggested that androgen treatments block FSH-induced increases in LH receptors in the rat. Conceptually, although large antral follicles are present during the luteal phase of the bovine, it is possible that P_4 or androgens may block the ability of these follicles to respond to gonadotropins prior to the gonadotropin surge. Similar observations were alluded to by Ireland and Roche (1983). No data on oestrogen concentrations for animals used in this study are available. Based on the follicular dynamics of cows examined, the dominant follicle present on day 14 was more likely a remnant of the day 7 dominant follicle. If such was the case, then it is possible that the follicles could have been regressing.

The induction of accessory CL during late dioestrus might have deprived the cows of the one follicle that could have secreted sufficient oestrogen to initiate the cascade of events leading to luteolysis. Luteinization of follicles is tantamount to destruction of the same. Fogwell et al., (1985) showed that cauterization of follicles extended the length of the cycle.

Another hypothesis suggests that hCG injections may inhibit oestrogen production by causing the depletion of the aromatizable substrates (Dieleman et al., 1983) or even barring the transfer of thecal androgens to the granulosa cells (Evans et al., 1981). It has also been proposed that hCG may decrease the aromatizing

capability of the granulosa membrane (Fortune and Hansel, 1979). If these hypotheses hold true, then it would be reasonable to assume that extensions in cycle length that have been reported, may be due to the effect of hCG either acting directly on the follicle or on its capability to synthesize steroids. These hypotheses are corroborated by evidence supplied by Howard and Britt, (1989) who reported that the presence of oestrogens is essential for increasing the sensitivity of the CL to prostaglandins. The above mentioned authors also noted no apparent changes in $PGF_{2\alpha}$ secretion.

Is it possible that the effect of hCG on cycle length is mediated via morphological changes in the various types of cells making up the CL (Wiesak, 1989). Treatments administered during early dioestrus (days 3-7) post oestrus reduce the size of the small luteal cells while decreasing, concomitantly, the number of large luteal cells (Bennett et al., 1989). Such an effect on the number of the large luteal cells would potentially attenuate the effect of $PGF_{2\alpha}$. These cells possess receptors to $PGF_{2\alpha}$ as a result reducing their numbers will jeopardise the process of luteolysis. But do these morphological changes take place in induced CL? Evidence of such responses is a subject that awaits future research.

It may be due to these various factors that there appears to be lack of unanimity on the effect of hCG on cycle length in the bovine. This extension varies depending on the timing, the dose used and the frequency of administering the treatments.

8. SUMMARY AND CONCLUSIONS

This study has contributed to the understanding of CL function in the Holstein dairy cow. We have demonstrated, with the aid of information available in the literature, that the dominant follicle present during the luteal phase of a cow is capable of ovulating. This observation supports the results obtained by Price and Webb, (1989). While the technique employed to verify the effect of hCG by Price and Webb, (1989) did not allow them to differentiate the induced structures from the spontaneous ones, use of an ultrasound machine in the present study facilitated our capability to identify the two types of CL. That technique and timing available for inducing the development of accessory CL repaid our efforts in two ways.

First, P_4 concentrations were increased in animals treated with hCG and one can only assume that the magnitude of the increaments were much more pronounced at the utero-ovarian vein. Animals showing the best response also showed increase even in pregnancy rates. This was more so among treatments rendered on day 7 post oestrus, a time identified as an appropriate window for supplementing endogenous P_4 production. Based on this type of response, we derive second conclusion. The initial assumption was that low P_4 concentrations which were indicative of CL dysfunction, accounted for a considerable proportion of the embryos which perished during the first 30 days of gestation. Such increases as we have shown confirm the fact that deficiencies in P_4 are, indeed, a legitimate cause of losses in fertility and that when the appropriate therapeutic measure is given at the correct time, we can minimize the incidence of such losses. The implications of

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these findings might find wide application not only in dairy cattle but also in embryo transfer programme.

These conclusions do not, in any way, rule out the involvement of other factors that have been identified to be predisposing agents in the demise of early embryos. It remains to be established for example how many of the embryos which were assisted to survive to term were initially genetically abnormal and would have therefore died of such a cause. Only time can answer such questions. We will count on time for the resolution of the functional capacity of the induced CL and effect of such treatments on follicular dynamics subsequent to the treatments.

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Appendix 1. Analysis of variance for Corpus Luteum diameter among animals receiving hCG either on day 0, 7, or 14 post breeding in comparison to control dairy cows at UBC South Campus.

(a) Dependent Variable : CL Diameter

Source of Variance	df	Sum of Squares	Mean Square	F Value	Inference
Model	51	29049.60	569.60	3.86	**
Treatment	3	7017.54	2339.18	3.95	*
Cow(trt)	28	16587.28	592.40	4.01	**
Sample	5	568.38	113.68	0.77	ns
Sam X Trt	15	2899.25	193.28	1.31	ns
Error	95	14019.06	147.57		
Total	146	43068.67			

N.B.: Values used in the above analysis were on data obtained on days 7, 14, 21, 28, 35 and 42, inclusive.
*   =   Significant at $P<0.05$
** =   Significant at $P<0.01$
ns =   Not Siginificant $P<0.05$
R^2 =   67.44 %

(b) Dependent Variable : CL Diameter

Source of Variance	df	Sum of Squares	Mean Square	F Value	Inference
Model	33	15682.66	475.23	7.07	**
Treatment	2	2642.78	1321.39	3.29	*
Cow(trt)	22	8836.99	401.68	5.98	**
Sample	3	471.79	157.26	2.34	ns
Sam X Trt	6	1788.60	298.10	4.44	**
Error	52	3494.65	67.20		
Total	85	19177.30			

N.B.: Values used in the above analysis were on data obtained on days 9, 11, 16 and 18, inclusive.
*   =   Significant at $P<0.05$
** =   Significant at $P<0.01$
ns =   Not Significant $P<0.05$
R^2 =   81.78 %

Appendix 2.   Analysis of variance for Plasma Progesterone concentration among animals receiving hCG either on day 0, 7, or 14 post breeding in comparison to control dairy cows at UBC South Campus.

(a) Dependent Variable : Plasma Progesterone

Source of Variance	df	Sum of Squares	Mean Square	F Value	Inference
Model	52	1092.88	21.02	3.75	**
Treatment	3	91.05	30.35	1.55	ns
Cow(trt)	29	567.82	19.58	3.50	**
Sample	5	142.01	28.40	5.07	**
Sam X Trt	15	96.56	6.44	1.15	ns
Error	97	543.36	5.60		
Total	149	1636.24			

N.B.: Values used in the above analysis were on data obtained on days 7, 14, 21, 28, 35 and 42, inclusive.
* = Significant at $P<0.05$
** = Significant at $P<0.01$
ns = Not Significant $P<0.05$
R^2 = 66.79 %

(b) Dependent Variable : Plasma Progesterone

Source of Variance	df	Sum of Squares	Mean Square	F Value	Inference
Model	33	585.84	17.75	2.87	**
Treatment	2	40.90	20.45	1.05	ns
Cow(trt)	22	427.22	19.42	3.14	**
Sample	3	72.42	24.14	3.91	**
Sam X Trt	6	17.94	2.99	0.48	ns
Error	59	364.67	6.18		
Total	92	950.51			

N.B.: Values used in the above analysis were on data obtained on days 9, 11, 16 and 18, inclusive.
* = Significant at $P<0.05$
** = Significant at $P<0.01$
ns = Not Significant $P<0.05$
R^2 = 61.63 %

Appendix 3. Analysis of variance for Milk Progesterone concentration among animals receiving hCG either on day 0, 7, or 14 post breeding in comparison to control dairy cows at UBC South Campus.

(a) Dependent Variable : Milk Progesterone

Source of Variance	df	Sum of Squares	Mean Square	F Value	Inference
Model	52	5682.37	109.28	3.49	**
Treatment	3	750.83	250.28	2.02	ns
Cow(trt)	29	3589.13	123.76	3.95	**
Sample	5	535.66	107.13	3.42	**
Sam X Trt	15	451.41	30.09	0.96	ns
Error	103	3229.54	31.35		
Total	155	8911.91			

N.B.: Values used in the above analysis were on data obtained on days 7, 14, 21, 28, 35 and 42, inclusive.
* = Significant at $P < 0.05$
** = Significant at $P < 0.01$
ns = Not Significant $P < 0.05$
R^2 = 63.76 %

(b) Dependent Variable : Milk Progesterone

Source of Variance	df	Sum of Squares	Mean Square	F Value	Inference
Model	26	2381.75	91.61	4.50	**
Treatment	2	650.08	325.04	3.71	*
Cow(trt)	15	1315.80	87.72	4.31	**
Sample	3	105.28	35.09	1.73	ns
Sam X Trt	6	43.64	7.27	0.36	ns
Error	39	793.11	20.34		
Total	65	3174.87			

N.B.: Values used in the above analysis were on data obtained on days 9, 11, 16 and 18, inclusive.
* = Significant at $P < 0.05$
** = Significant at $P < 0.01$
ns = Not Significant $P < 0.05$
R^2 = 75.02 %

Appendix 4.   Analysis of variance for Milk Progesterone concentration among animals receiving hCG either on day 0, 7, or 14 post breeding in comparison to control dairy cows at Farm # 1.

(a) Dependent Variable : Milk Progesterone

Source of Variance	df	Sum of Squares	Mean Square	F Value	Inference
Model	76	9188.83	120.91	6.15	**
Treatment	3	603.18	201.06	2.27	ns
Cow(trt)	49	4339.70	88.57	4.50	**
Sample	6	3593.73	598.96	30.45	**
Sam X Trt	18	521.41	28.97	1.47	ns
Error	257	5055.91	19.67		
Total	333	14244.74			

N.B.: Values used in the above analysis were on data obtained on
      days 0, 7, 9, 11, 14, 16 and 18, inclusive.
* = Significant at P<0.05
** = Significant at P<0.01
ns = Not Significant P<0.05
R^2 = 64.51 %

(b) Dependent Variable : Milk Progesterone

Source of Variance	df	Sum of Squares	Mean Square	F Value	Inference
Model	53	4486.58	84.65	2.80	**
Treatment	3	102.55	34.18	0.35	ns
Cow(trt)	38	3697.47	97.30	3.22	**
Sample	3	101.84	33.95	1.12	ns
Sam X Trt	9	115.34	12.82	0.42	ns
Error	50	1510.02	30.20		
Total	103	5996.59			

N.B.: Values used in the above analysis were on data obtained on
      days 21, 28, 35 and 42, inclusive.
* = Significant at P<0.05
** = Significant at P<0.01
ns = Not Significant P<0.05
R^2 = 74.82 %

Appendix 5. Analysis of variance for Milk Progesterone concentration among animals receiving hCG either on day 0, 7, or 14 post breeding in comparison to control dairy cows at Farm # 2.

(a) Dependent Variable : Milk Progesterone

Source of Variance	df	Sum of Squares	Mean Square	F Value	Inference
Model	111	27961.38	251.90	7.12	**
Treatment	3	1570.95	523.65	5.05	**
Cow(trt)	84	8712.28	103.72	2.93	**
Sample	6	8263.46	1377.24	38.93	**
Sam X Trt	18	1953.64	108.54	3.07	**
Error	344	12169.66	35.38		
Total	455	40131.03			

N.B.: Values used in the above analysis were on data obtained on days 0, 7, 9, 11, 14, 16 and 18, inclusive.
* = Significant at $P<0.05$
** = Significant at $P<0.01$
ns = Not Significant $P<0.05$
R^2 = 69.68 %

Appendix 6a.   Number of animals (n) used in the statistical analyses at each respective time following breeding at the UBC South Campus Farm.

	Treatment			
	Day 0	Day 7	Day 14	Control
Time Post AI				
7	7	9	9	7
9	7	9	9	7
11	7	9	9	7
14	6	9	9	7
16	6	9	9	7
18	5	7	9	7
21	4	7	6	3
28	3	6	6	3
35	3	6	5	3
42	3	6	5	3

Appendix 6b.   Number of animals (n) used in the statistical analyses at each respective time following breeding on Farm # 1.

	Treatment			
	Day 0	Day 7	Day 14	Control
Time Post AI				
0	19	20	20	19
7	19	20	20	19
9	19	20	20	19
11	19	20	20	19
14	18	20	20	18
16	16	20	20	17
18	14	20	20	17
21	9	14	14	12
28	7	14	10	9
35	7	14	9	8
42	7	14	9	7

Appendix 7.   Number of animals (n) used in the statistical
analyses at each respective time following breeding on Farm # 2.

	Treatment			
	Day 0	Day 7	Day 14	Control
Time Post AI				
0	23	27	24	26
7	23	27	24	26
14	21	26	23	26
21	12	23	18	15
28	12	21	12	13
35	10	19	12	13
42	10	19	12	12