EFFECTS OF HUMAN CHORIONIC GONADOTROPIN STIMULATION ON CORPUS LUTEUM FUNCTION AND FERTILITY IN CATTLE

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DEDICATION

This thesis is dedicated to my parents, Erhirhie and Omeke Eduvie, my grandmother Etenure Akpojivi, my uncles Moses and Michael Akpojivi, and to my sisters and brothers, for their love and the parts they played in my educational career.

TABLE OF CONTENTS

	Page
LIST OF TABLES	iv
LIST OF FIGURES	v
INTRODUCTION	1
REVIEW OF LITERATURE	4
Effects of HCG on the life span of the CL	5
Effects of HCG on *in vitro* and *in vivo* progesterone synthesis	8
Effects of HCG on CL size and weight	12
Formation of antibodies against HCG	12
Effects of HCG on fertility	14
MATERIALS AND METHODS	16
Animals and Experimental Design	16
Part 1: Effect of HCG on CL function	16
Palpation and blood collection	16
Heat detection	19
Radioimmunoassay of progesterone	20
Determination of antibody formation against HCG	25
Part 2: Fertility trial with HCG	28
Statistical Analysis	29
RESULTS	30
Part 1: Effect of HCG on CL function	30
Part 2: Fertility trial with HCG	42

Page

DISCUSSION..... 48

SUMMARY AND CONCLUSIONS..... 55

BIBLIOGRAPHY..... 57

ACKNOWLEDGEMENTS..... 68

LIST OF TABLES

1 HCG treatment schedule

2 Distribution of cycle lengths of control and treated cows (Part 1)

3 Effect of HCG injections on serum progesterone

4 Conception rates of heifers and cows treated with HCG 10-12 days after breeding

5 Distribution of cycle lengths (up to 40 days) of bred but non-pregnant cows (Part 2)

LIST OF FIGURES

1 Sample of an Ouchterlony plate

2 Average cycle lengths and serum progesterone levels for control cows and cows given HCG every 7 days (group A) or 14 days (group B) beginning on day 9, 10 or 11 of the estrous cycle

3 Effect of HCG injections on progesterone levels and estrous cycle lengths of individual cows given HCG every 7 days (group A) beginning on day 9, 10 or 11 of the estrous cycle

4 Effect of HCG injections on progesterone levels and estrous cycle lengths of individual cows given HCG every 14 days (group B) beginning on day 9, 10 or 11 of the estrous cycle

INTRODUCTION

It is now known that one of the most common conditions causing infertility in dairy cattle is that of ovarian cystic degeneration. McKay and Thomson (1959) reported that 12 to 14% of problem breeder cows had cystic ovaries. Zemjanis (1970) reported 5.7% incidence of ovarian cysts in about 21,000 postpartum dairy cows. Roberts (1971) defined a follicular cyst as an anovulatory follicle that persists on the ovary for 10 days and usually much longer, has a diameter greater than 2.5 cm and is characterized by nymphomania, continuous or frequent estrus, or by anestrus. A luteal cyst is also an anovulatory follicle over 2.5 cm in diameter, but is partially luteinized, persists for a prolonged period and is usually characterized by anestrus.

The actual mechanism leading to the development of ovarian cystic degeneration is not completely known. However, reports of successful treatment of this condition with gonadotropic substances rich in luteinizing hormone (LH) indicate that the basic cause is a failure of the hypophysis to release sufficient amounts of LH to effect ovulation and proper development of the corpus luteum (CL). Casida, McShan and Meyer (1944) reported successful treatment of many cows with ovarian cystic degenerations both follicular and luteal, using unfractionated sheep pituitary extracts intravenously. Jubb and McEntee (1955) and McEntee (1958) reported that the delta cells of the anterior pituitary which produce and store gonadotropic hormones degranulate or release their hormone shortly after the onset of estrus

in normal cows. In cows that failed to ovulate by day 3 after estrus, they found this degranulation had not occurred. Donaldson and Hansel (1968) reported that pituitary gonadotropin potency at estrus and 4 days after normal estrus and ovulation ranged from 3.0 to 9.1 μg while in cows with follicular cysts, the pituitary glands contained 6.1 to 15.1 μg of gonadotropin at estrus and 4 days later. This was interpreted as a failure of normal release of LH which prevented normal ovulation and development of the CL.

Thus the treatment of ovarian cystic degeneration involves making LH available to the granulosa cells of the ovarian follicle to effect luteinization and subsequent reestablishment of the estrous cycle. Human chorionic gonadotropin (HCG) has been widely used for the treatment of ovarian cystic degeneration since the recognition of its LH activity (Mason et al, 1962; Roberts, 1971). As the possibility of misdiagnosis of this condition by rectal palpation cannot be ruled out, the main objective of this study was to determine the effect of repeated administration of this drug on CL function and estrous cycle duration in normal cows.

The possible use of HCG as a method of improving conception rate based on its ability to induce ovulation and enhance CL development has been tested (Hansel et al, 1960 and 1976; Brown et al, 1973; Morris et al, 1976). However, the effect prolongation of CL function with HCG might have on fertility is not known. It seems reasonable to think that by extending CL function and delaying the subsequent estrus, the fertilized egg or embryo would have a greater chance

than otherwise, to become established in the uterus and inhibit the luteolytic factor. Also, by delaying the next estrus, some early embryonic deaths might be prevented. Hence a second objective of this study was to test the effect prolongation of CL function with HCG given 10-12 days after artificial insemination might have on fertility rate.

REVIEW OF LITERATURE

Human chorionic gonadotropin (HCG) is a glycoprotein hormone which has biologic activity similar to luteinizing hormone (LH) and appears to be responsible for maintenance of the corpus luteum (CL) of gestation (Niswender et al, 1972). This hormone has been shown to stimulate the interstitial cells of the ovary, cause ovulation, bring about luteinization of the granulosa cells, maintain the functional life of the CL and increase progestin secretion from luteinized cells (Aschheim and Zondek, 1927; Cole, 1969; Neil and Krobil, 1972; Channing and Kammerman, 1973).

Simmons and Hansel (1964) demonstrated the luteotropic properties of HCG when they succeeded in overcoming the inhibitory effect of oxytocin on the CL with injections of various levels of HCG on different days of the estrous cycle into heifers. Wiltbank and co-workers (1961), Moody and Hansel (1971) and Veenhuizen and co-workers (1972) also reported the luteotropic effect of repeated HCG administration on the CL.

The CL, compared with other endocrine glands, is a transitory structure with a short but variable life span. It could be theoretically influenced by at least 2 different types of trophic stimuli, the 1 increasing its secretory activity and the other prolonging its life span.

Effects of HCG on the life span of the CL

Several workers have reported increases in the life span of the CL, and thus extension of the diestrual period of the estrous or menstrual cycle, by injections of HCG or pregnancy urine extracts in various species.

Specifically, Katzman et al (1931) prolonged the diestrual period of adult rats with daily injections of 0.03 to 0.001 mgm of pregnancy urine extracts. A similar administration of pregnancy urine extracts (100 mouse units daily) into women after the beginning of the luteal phase increased the total amount of pregnanediol excreted and extended the luteal phase to 17 days, with the onset of the next bleeding correspondingly delayed (Browne and Venning, 1938).

Hisaw (1944) reported extension of the life of the CL of rhesus monkeys with daily injections of chorionic gonadotropin (Prolan B, Pu) beginning between day 18 and day 24 of the menstrual cycle, while McDonald (1971) treated monkeys on days 10-13 of the cycle with anti-HCG serum and blocked ovulation, decreased luteal phase progesterone levels and shortened the length of the cycle.

Brown and Bradbury (1947) observed that injections of 10,000 or 20,000 IU HCG given daily to women beginning in the late secretory phase of the cycle delayed the menstrual period by 12 or 19 days accompanied by decidual changes in the endometrium. When the initial chorionic gonadotropin treatment was given very late in the secretory phase, that is, within 24 hours of the onset of bleeding, no effect was obtained indicating the hormone was unable to revive an involuting CL.

Beginning early in the postovulation period of the menstrual cycle, Segaloff et al (1951) treated 6 women with 10,000 IU HCG intramuscularly (IM) and noted a significant delay in the onset of the next menstrual period.

Similarly, Bryans (1951) prolonged the menstrual cycle of 2 monkeys 7 and 10 days beyond the expected normal duration with daily subcutaneous (SQ) injections of 1000 IU HCG commencing from day 10 and 22, respectively.

Using a combination of 2000 IU HCG and 200 units of prolactin administered every other day starting within a week following the ovulatory temperature in women, Fried and Rakoff (1952) prolonged CL function for various periods depending on duration of therapy. Like some previous workers, they failed to revive or maintain regressing CL by this therapy.

In an experiment to determine the effect of daily injections of HCG on the maintenance of the CL and on embryo survival in the cow, Wiltbank et al (1961) observed that the estrous cycles of 5 heifers were prolonged from an average of 17.7 days to 32.4 days by daily IM injections of 1000 IU HCG from day 15 to day 26 (estrus = day 1) of the cycles. The CLs were maintained in these heifers as well as in bred heifers which were subsequently diagnosed open.

Donaldson and Hansel (1965) reported mean estrous cycle lengths of 31 and 36.4 days for 2 groups of 4 cows that were given pituitary extract and bovine LH in Freund's complete adjuvant respectively on day 16. The average length of the control cycles was 20 days. The subsequent cycles in all the cows were normal

except for 1 LH-treated cow that had a 14- followed by an 11-day cycle.

Strott et al (1969) prolonged the luteal phase of the menstrual cycle of women from 7 to 21 days when 2000 IU HCG were administered daily for 10 days beginning 4 days after "Mittelschmerz" (abdominal pain occurring at time of ovulation). They found no difference in cycle lengths between control and treated when HCG administration was started at the estimated time of ovulation. Ginther (1970) reported increased cycle length in ewes given estradiol plus HCG and HCG alone on day 8 through 11.

A continuous intravenous (IV) infusion of purified ovine LH commencing on day 10-12 prolonged the life span of the CLs of ewes to arbitrarily chosen times of 20, 25 or 30 days in 62% of the test animals (Karsch et al, 1971). This infusion was, however, less effective in maintaining the CLs to day 20 when begun on days 13 and 14 and totally ineffective when initiated on day 15. The studies of Hanson, Powell and Stevens (1971) showed that treatment of normally cycling women during the luteal phase of the cycle with LH or HCG extended the life of the CL.

In their experiment to determine the luteotropic effect of HCG in heifers, Seguin et al (1977) prolonged CL function by approximately 7 and 5 days with single IM injections of 10,000 IU HCG given on day 10 and 15, respectively. Injections of HCG on day 17 of the estrous cycle had no effect.

Effects of HCG on *in vitro* and *in vivo* progesterone synthesis

Evidence exists from both *in vitro* incubation and perfusion studies that LH or HCG can increase the rate of progesterone synthesis by the luteal tissue of the cow (Savard and Casey, 1964; Hall and Koritz, 1965; Savard, Marsh and Rice, 1965; Armstrong and Black, 1966; Hansel, 1966; Bartosik and Romanoff, 1971). When administered concurrently with oxytocin into heifers from day 0 through day 10, HCG was able to overcome the inhibitory effects of oxytocin on CL progesterone content and concentrations (Simmons and Hansel, 1964). Inactivation of the LH component of the HCG by incubation with 6M-urea at 40° C for 24 hours (Schmidt-Elmendorff, Loraine and Bell, 1962) abolished its ability to increase CL progesterone content and concentration in the oxytocin treated animals.

Mason et al (1962) observed that HCG caused a 2- to 3-fold stimulation of progesterone biosynthesis when added in the concentration range of 70 and 4000 IU per gram of bovine luteal tissue while Rice et al (1964) reported 100% and 400% increase in the formation of progesterone by addition of 10 and 100 IU HCG, respectively.

Armstrong et al (1964), and Armstrong and Black (1966) demonstrated the ability of gonadotropic hormone preparations to stimulate progesterone synthesis in CL slices of the rat and dairy cow, respectively. Seifart and Hansel (1965) had earlier observed an increase in the net *in vitro* progesterone synthesis in incubated slices of bovine luteal tissue by the addition of purified bovine LH to the incubated medium.

Romanoff (1966) noted that NIH-LH-S^4 caused consistent increases in the rate of synthesis of progesterone when bovine luteal ovaries were perfused. Similar stimulation of progesterone synthesis by LH added to *in vitro* incubated luteal tissue slices have also been demonstrated in luteal tissues of the rabbits (Gorski et al, 1965), pig (Cook et al, 1967), sheep (Kaltenbach et al, 1967) and the opossum (Cook and Nalbandov, 1968).

In their investigation of progesterone synthesis *in vitro* by human CLs during the reproductive cycle, LeMaire et al (1968) found a significant increase in the level of progesterone after incubation with LH or HCG. Also working on progesterone biosynthesis by human CLs *in vitro*, Maeyama et al (1970) reported that the net *de novo* synthesis of progesterone by the CLs was significantly increased by the addition of 500 IU HCG.

While 500 IU HCG also stimulated *in vitro* steroidogenesis leading to increased progesterone synthesis from porcine ovarian tissue (Aakvagg, 1970), addition of only 1.0 IU HCG to incubated bovine CL also increased the progesterone concentration 5-fold (Veenhuizen et al, 1972). Channing and Kammerman (1972) added HCG to monkey granulosa cells cultured for 16-20 days and reported luteinization of granulosa cells, maintenance of the functional life of the CL and increased progestin secretion from the luteinized cells.

Several workers have observed significant effects of HCG on *in vivo* progesterone synthesis and secretion in different species. A single IV injection of 0.5 IU HCG resulted in a prompt and remarkable

increase in the concentrations of progesterone, 5_{α}-pregnane-3, 20-dione and 3_{α}-hydroxy-5_{α}-pregnan-20-one in the ovarian venous plasma of proestrus rats (Ichikawa et al, 1972). Shaikh and Harper (1972) observed peak steroid concentrations in rabbit ovarian vein blood at 2 1/2-3 hours after HCG (200 IU) administration and a second dose to the HCG treated rabbits gave further stimulation to progestin output with little effect on estrogen secretion.

Morris et al (1976) injected 1000 IU HCG IV into heifers 96 hours after estrus, and observed an increase in serum progesterone from 2.0 ng/ml in the control heifers to 3.1 ng/ml in the treated heifers on days 9 and 16 of the cycle. Average progesterone levels increased following treatment of lactating cows for ovarian cysts with either 50-250 μg GnRH or 10,000 IU HCG (Seguin et al, 1976; Garverick et al, 1976). Schomberg et al (1967), however, reported a variable effect of HCG on progesterone secretion following single intravenous administration of 5000 IU on days 5, 10, 15 and 20 of the estrous cycle. The HCG treatments caused a significant increase in the blood progesterone concentrations of 1 cow on day 5; in contrast, treatments on days 10, 15, and 20 had no effect. In another cow HCG did not produce a significant increase in plasma progesterone on day 5, but significant responses were produced on days 10, 15 and 20.

Daily administration of HCG (17.5 to 500 IU) to monkeys beginning on days 21 to 24 of the menstrual cycle, when plasma progesterone concentrations were falling in the normal fashion, resulted in sharp increases in circulating progesterone levels which

were maximal in the first post injection sample taken 24 hours after the first hormone injection (Neil and Krobil, 1972). Also, daily injections of 50 IU HCG to monkeys starting from the 10th day following the midcycle estrogen peak (Surve et al, 1973) and of 1000 IU from day 1 postpartum to day 4 (Stouffer et al, 1976) significantly increased blood progesterone levels above those of the controls.

Injections of 1500-3000 IU HCG daily or every other day during the luteal phase of the menstrual cycle produced a 2-3 fold plasma progesterone increase in women (Strott et al, 1969; Hansel et al, 1971). To determine the effect of HCG on CL function during the puerperium, LeMaire et al (1971), infused (IV) into women, 40,000 to 90,000 IU HCG per day continuously for 5 days beginning immediately after delivery at term. This markedly altered the rate of decline of HCG levels and increased CL biosynthesis of progesterone. A similar infusion, also in women, of 50,000 IU HCG for 24 hours following termination of pregnancy delayed the decline of serum HCG levels and maintained 17-OHP (17_{α}-hydroxy progesterone) levels at preoperative levels for the 24 hours of the infusion (Garner and Armstrong, 1977). Stock et al (1971) observed that treatment of women with HCG (10,000 IU) every other day during the luteal phase, resulted in an increased ovarian hormone excretion and the steroid levels remained elevated while the onset of menstruation was postponed by 7-14 days.

Runnebaum et al (1972) infused (IV) 5000 IU HCG and 20,000 IU HCG into women on day 5 of the menstrual cycle and increased peripheral plasma progesterone by 70% and 250%, respectively.

Effects of HCG on CL size and weight

Reports on increases in size and weight of CLs from cows treated with HCG are other evidences of the luteotropic properties of this hormone. Total doses of 15,000 and 13,000 IU HCG on days 1 and 7 of the estrous cycle produced 16.4 and 10.8 gm corpora lutea respectively, while a dose of 5000 IU on day 6 followed by 2 mg estradiol 8 hours before removal of the CL on day 11 yielded 12.1 gm CL compared to 5.8 gm average for 2 control heifers (Moody and Hansel, 1971) and the 5.6 gm average for normal day 11 corpora reported by Simmons and Hansel (1964).

Veenhuinzen et al (1971) also reported significant increases in bovine CL size after daily HCG administration from day 2 to day 7 as well as after single injections (1000 IU) on either day 1 or 3 of the estrous cycle. Daily injections of 1000 IU HCG from day 1 post-partum to day 4 resulted in corpora lutea of greater weights than those from control monkeys (Stouffer et al, 1976).

Formation of antibodies against HCG

Repeated administration of HCG which is a heterologous protein hormone with a molecular weight of about 40,000 to mammals frequently leads to the loss of responsiveness to this hormone.

A progressive decline in the number of eggs shed and CLs formed after multiple injections of superovulatory doses of HCG or pregnant mare serum gonadotropin (PMSG) has been reported in several species (Parkes, 1942, Mauer et al, 1968 in the rabbit; Willett et al, 1953 in the cow; Edwards and Fowler, 1960, Lin and Bailey, 1965 in the

mouse). Parkes (1942) observed that a 10-day treatment of adult albino does with horse pituitary extract caused a decrease in ovarian stimulation, suggesting antibody formation.

Further evidence of immunity to gonadotropin was indicated by ovulatory failures observed in several species after repeated gonadotropin treatment (Adams, 1953, 1961 and Greenwald, 1970 in rabbit; Land and McLaren, 1967 in mice; McDonald, 1971 in monkey). Thompson (1941) had earlier concluded from his experiments that ovulatory refractoriness was due to the build-up of an immunity to exogenous hormones. Meckley and Ginther (1972) reported shortening the estrous cycle of guinea pigs with injections of HCG on days 2, 3 and 4. Reel et al (1976) demonstrated ovulatory refractoriness to HCG in the rabbit following cyclic injections of 50-100 IU HCG at 3-week intervals for 1-18 cycles.

Antihormones and antibody titers against the combination of sheep anterior pituitary extract and HCG (Synapoidin®) were found to develop readily in the rabbit (Chase, 1945; Leathem, 1945, 1946). Rakoff and Leathem (1946) indicated that antihormone formation may occur following protracted treatment of humans with a combination of sheep anterior pituitary extracts and HCG. High serum titers of anti-HCG were observed following chronic treatment of mares (Snook and Cole, 1964) and ewes (Snook et al, 1971) with HCG. A decline in steroid excretion and eventual cessation of menstruation in spite of continued HCG treatment (10,000 IU given every other day during 11 luteal phases) have been reported in women (Stock et al, 1971).

Hoffmann et al (1973) inhibited CL function in the cow with LH antiserum while Thompson (1939) caused a developmental disturbance of the reproductive system of young rats by injecting a non-species-specific canine gonadotropic antihormone which was also capable of terminating pregnancies of dogs within 100 hours of the first injection. A reduction in the number of mice that mated and ovulated after more than 1 injection of PMS-HCG compared to those that received 1 treatment (Fowler and Edwards, 1957; Edwards and Fowler, 1958), is another evidence of antigonadotropin formation following its repeated administration.

Effects of HCG on fertility

The reports on the effects of HCG on fertility are rather conflicting. While some workers have observed some improvements in conception rates following use of HCG at breeding, others have recorded no difference or lowered conception rates.

Human chorionic gonadotropin (2000 IU) injected into heifers (SQ) at the time of insemination increased pregnancy rate by 15%, points at both the first and second synchronized breeding periods, but reduced pregnancy rate when given 3 days after insemination (Wagner et al, 1973). A single (SQ) injection of 1500 IU HCG at the time of insemination also increased pregnancy rates in synchronized and non-synchronized heifers (Brown et al, 1973). Babler and Hoffman (1974) gave 1500 IU HCG in 5% beeswax to dairy cows and obtained a conception rate of 78.7% compared to 63.8% rate for control animals. Earlier, Palsson (1962) reported that fertility in ewes was

substantially increased under farming conditions by the injection of PMS on the 12th or 13th day of the last estrous cycle before mating. Lehnert and Dukelow (1977) concluded from their study, that HCG (10 IU or 100 IU) given to young adult hamsters on days 6-11 of gestation, exerts a positive effect on litter size.

Administration of 2000 IU HCG to repeat breeder cows did not improve conception rate (Hansel et al, 1960), while a dose of 1500 IU at the time of insemination was not effective in stimulating conception rates in lactating dairy and beef cows under field conditions (Hansel et al, 1976). Morris et al (1976) found no effect on fertility when heifers were given 1000 IU HCG 96 hours after breeding. Sullivan et al (1973) and Voss et al (1975) shortened the duration of estrus of mares by 3.1 days with an IM injection of 2000-3300 IU HCG given during estrus 24 hours before the first insemination but there was no difference in pregnancy rate between treated and control animals. A daily administration of 1000 IU HCG to heifers from the 15th day to 35th day after breeding did not significantly increase the conception rate (Wiltbank et al, 1961). However, ovarian activities increased as evidenced by development of accessory corpora lutea and big follicles in the HCG-treated heifers.

MATERIALS AND METHODS

This study was carried out in 2 parts. Part 1 involved repetitive administration of HCG into normally cycling non-bred diestrous cows to determine its effect on CL function. In part 2, a fertility trial with HCG was conducted on dairy farms to determine the effect prolongation of the diestrual period of the cycle following breeding might have on conception rate.

Animals and Experimental Design

Part 1: Effect of HCG on CL function

Twenty-two mature dairy cows weighing approximately 550 kg each were used in this experiment. These animals were housed in the University campus dairy barn and fed a balanced dairy ration.

Ten cows served as controls while the remaining 12 were randomized into 2 groups (group A and B) of 6 each. All the cows in groups A and B were given IM injections of 10,000 IU HCG (Table 1) (Chorionatrop[1]): Group A every 7 days and B every 14 days beginning from day 9, 10 or 11 of the estrous cycle (estrus = day 0). All cows in group A received a total of 4 injections each, except 1 (cow no. 886) that came in heat shortly after the third injection. Each cow in group B got 2 injections. The control cows were given 10 ml isotonic saline solution (IM) on day 9, 10 or 11.

Palpation and blood collection

The reproductive tracts were palpated per rectum, at least twice a week to determine the status of the CLs and stage of the cycle

[1] Biological Specialties, Middleton, WI 53562.

TABLE 1

HCG treatment schedule

Treatments	Day of cycle when treated[a]			
	#1	#2	#3	#4
Group A	9,10,11	16,17,18	23,24,25[b]	30,31,32
Group B	9,10,11	23,24,25[b]	-	-

[a] 10,000 IU HCG IM.

[b] Estrus at day 21 was not anticipated.

(Zemjanis, 1970). Blood was collected every 3 days or more frequently, depending on the status of the CL, by tail venipuncture with 20 gauge 1 1/2" vacutainer[2] needles into 10 ml vacutainer tubes. The blood was allowed to clot at room temperature (1-2 hours), then transferred into a cold room at 4° C to stay overnight. After centrifugation for about 10-15 minutes, serum was then collected and stored at -20° C until assayed for progesterone by radioimmunoassay (RIA) (Garza et al, 1975). Some selected serum samples (i.e. samples collected about 2 weeks after the last HCG injection) were also used for determination of antibody formation against HCG (Ouchterlony, 1958).

Heat detection

The animals were let out for 30-45 minutes every morning during which time they were closely watched for signs of heat. A cow that stood to be mounted by another cow and had a palpable follicle plus increased uterine tone was regarded as being in heat.

Palpation, blood collection and heat detection were continued during the next cycle after treatment to determine the effect of the treatments on subsequent cycles.

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[2] Vacutainer® System, Becton-Dickinson and Co., Rutherford, NJ 07070.

Radioimmunoassay of progesterone

A radioimmunoassay (RIA) procedure similar to that reported by Garza et al (1975) and described in detail by Oyedipe (1978) was used to quantify progesterone in this study.

1. Assay ingredients:

   a. Assay buffer:

      Phosphate buffered saline gelatin[3] (PBSG) consisting of a mixture of 16.35 gm sodium phosphate dibasic heptahydrate ($Na_2HPO_4 7H_2O$), 5.4 gm sodium phosphate monobasic monohydrate ($NaH_2PO_4 H_2O$), 0.1 gm thimerosal,[4] 9 gm sodium chloride (NaCl) and 1 gm gelatin, was used as assay buffer.

   b. Assay and recovery tracers:

      Progesterone -1, 2, 6, 7 -3H (specific activity = 0.25 Ci/mmole) was obtained from New England Nuclear Corporation[5] and stored at 4° C in 5 ml benzene. An aliquot of this stock labelled progesterone was purified on a sephadex-LH-20[4] chromatography column by a method similar to that described by Carr et al (1971), taken up in 3 ml benzene and stored at 4° C. A fraction of this 'cleaned' tritiated progesterone was then dried under air, and PBSG was added to obtain the assay tracer ("1X") of a concentration of 10,000 cpm (counts per minute)/0.1 ml.

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[3] Difco Laboratories, Detroit, MI 48233.

[4] Sigma Chemical Company, St. Louis, MO 63178.

[5] New England Nuclear, Boston, MA 02218.

A further dilution of the "1X" to 1000 cpm/0.1 ml gave the recovery tracer.

c. Antibody:

The lyophilized progesterone antiserum S7#4 used, was developed by Abraham et al (1971) and obtained from Bar Harbor General Hospital, Torrance, California.[6] This was dissolved in 0.7 ml PBSG and stored at -70° C in 0.1 ml aliquots. An antibody dilution (avidity) curve was established by titration of serial dilutions of the antiserum (in PBSG) with labelled progesterone. The dilution that bound about 50% of the labelled hormone (1:550) was used for the assay. This antiserum has been shown to be specific to progesterone with minimal or no cross reaction with other steroids (Garza et al, 1975).

d. Progesterone standards:

Progesterone for standards was supplied by the Sigma Chemical Company.[4] The hormone was dissolved in ethanol to give a stock concentration of 1 μg/ml and stored at -20° C. Aliquots of this were taken and diluted with PBSG to concentrations of 10, 1 and 0.1 ng/ml buffer to make high, medium and low standard stock solutions, respectively.

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[6] Harbor General Hospital, Torrance, CA 90502.

e. Dextran-coated charcoal (DCC):

A dextran-coated charcoal (DCC) suspension consisting of 0.625% Norit A[4] charcoal and 0.0625% dextran T-70[7] in PBSG was used for the assays.

f. Scintillation fluid:

To make this, 50 ml PPO-POPOP concentrated liquid scintillator[8] and 198 ml paradioxane (scintillation grade) were added to 1 liter of toluene.[9]

2. Assay procedure:

Serum steroids were extracted twice with 4 mls of hexane (nonspectro)[10] from 0.5 ml of each of the serum samples assayed, and stored in 1 ml PBSG for at least 4 hours at 4° C. To determine the recovery of the tritiated tracer added prior to extraction, 0.2 ml of the extracted sample in duplicate, was added to 4 ml scintillation fluid and counted for 10 minutes (Beckman LS 330 Liquid Scintillation System).[11]

Two 0.2 ml aliquots of each of the extracted samples were added to test tubes containing 0.3 ml PBSG. To this a 0.1 ml of antiserum and a 0.1 ml of assay tracer ("1X") were added with vortexing between each addition. The tubes were covered

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[7] Pharmacia Fine Chemicals, Piscataway, NJ 08854.

[8] Research Products Int. Corp., Elk Grove Village, IL 60007.

[9] Fisher Scientific Company, Fair Lawn, NJ 07410.

[10] Burdick and Jackson Laboratories, Inc., Muskegon, MI 49442.

[11] Beckman Instruments, Inc., Fullerton, CA 92634.

and stored at 4° C for at least 2 hours (or overnight). DCC (0.2 ml) was then added to each tube, mixed and allowed to stand for 20 minutes before centrifugation at 4-5° C (10 minutes, x 1000g). The supernatants were decanted into vials containing 10 ml scintillation fluid, and counted. A standard curve was established for each assay using progesterone at levels of 10, 25, 50, 100, 250, 500 and 1000 pg by diluting aliquots of the standard progesterone stock solutions to 0.5 ml with PBSG. The tubes containing these standards as well as non-specific binding tubes (NSB) and maximum binding tubes (Bo) were all treated in the same manner as tubes containing the unknowns. For comparison among assays serum samples from a diestrus and an estrus cow as well as extracts from blank extraction tubes were assayed with each set of unknown serum samples. Triplicate total count tubes (TCT) containing 0.1 ml "1X" and 10 mls scintillation fluid were also prepared and counted. The antibody bound radioactivity in the Bo tubes was taken as 100% and tubes containing standards or unknowns were expressed as a percent of the Bo.

To plot the standard curve, the percent bound values obtained for standards were converted to logits $\left(\text{logit \% bound} = \frac{\log \text{ \% bound}}{100\% \text{ bound}}\right)$ and plotted against the standard progesterone values. This procedure converted the standard curve into a straight line. To obtain the unknown hormone

values, the % bound for each sample was determined, extrapolated from the standard curve and corrected for volume and recovery.

3. Assay validation:

Eighty percent of the tritiated tracer added to the serum samples prior to extraction was recovered in all the assays (i.e. 80% extraction efficiency).

Accuracy: Known concentrations of non-tritiated progesterone were added to assay buffer. These were extracted and assayed like the unknown samples; 98.1 $\pm$ 1.3% of this was measured.

Precision and sensitivity: The intra and inter assay coefficients of variation of replicate serum samples from a diestrous cow were 2.9% and 7.2% respectively, and the minimum detectable amount of progesterone in the assays was 0.1 ng/ml of serum. All buffer blank samples gave non-detectable results.

Determination of antibody formation against HCG

The double diffusion plate technique of Ouchterlony (1958) was used to determine antibody formation against the injected HCG.

Ingredients:

Plates:

To prepare the plates for this technique, 6 strips of Whatman No. 1 filter paper[12] (1 5/8" x 1/2") were folded so that 1 end lay flat at the bottom of the male half of a 90 mm petri dish with the other end hanging over the side. The strips were arranged evenly around the periphery of the petri dish and a 26 gauge stainless steel wire, 24 inches long, was placed inside the male half of the petri dish to hold the paper strips against the sides and the bottom.

Phosphate buffered saline:

Phosphate buffered saline was prepared by placing 2.6796 gm sodium phosphate dibasic heptahydrate ($Na_2HPO_4 7H_2O$) and 0.3233 gm potassium phosphate monobasic (KH_2PO_4) in a 1 titer volumetric flask and diluting to mark with 0.85% saline (34 gm NaCl made to 4 titers with distilled water). This was then transferred into a 2 1/2 liter storage bottle and 1475 mls of 0.85% saline was added.

Agar for Ouchterlony plates:

Ionagar[13] #2 (8.5 gm) and 0.1 gm merthiolate[14] were placed in a 2 liter flask and made up to 1 liter with phosphate buffered

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[12] W and R Balston Ltd., England.

[13] Consolidated Laboratories, Inc., Chicago Heights, IL 60411.

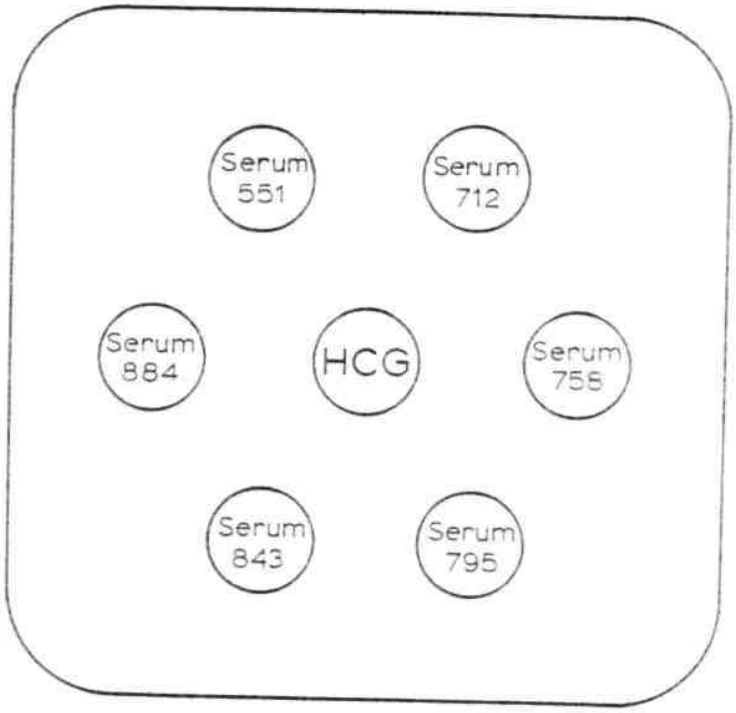
[14] K and K Laboratories, Plainview, NY 11803.

saline, autoclaved for 15 minutes at 15# pressure, and filtered hot through Whatman #41 filter paper[12] into another flask. Thirty mls of the agar was poured into each of the prepared petri dishes, allowed to form gels, then covered and stored for 24 hours at 4° C before use.

Well patterns were cut with Feinberg Agar Gel Cutter[13] in the agar and the cut-out pieces were melted and a few drops of this were added to each well (1 and 2 drops to outside and center wells, respectively) to seal the bottom. The center well was filled with about 13 drops of HCG (5000 IU/ml) and each of the outside wells with 5-8 drops of each serum sample and incubated at room temperature for 14 days in a humid chamber. The agar gel was then removed from the petri dish, soaked in 0.85% saline for 3 days and rinsed in distilled water. The remaining agar gel after cutting excess gel from the periphery, was placed on a 3 1/4" x 4" glass slide, and covered with 2 layers of Whatman No. 1 filter paper[12] moistened with distilled water; and allowed to dry overnight.

The agar was stained in Ouchterlony protein stain for 25 minutes, differentiated in 2% acetic acid for 5 minutes in each of 3 acetic acid dishes, and rinsed in distilled water for 15 minutes. It was allowed to dry and then examined for bands of antigen-antibody complex. Control sera obtained before HCG injections were also prepared for comparison.

Figure 1
Sample of an Ouchterlony Plate



Part 2: Fertility trial with HCG

Two hundred cows and heifers in 9 different herds were used in this trial. As cows observed in estrus were assigned to be bred they were assigned to control and treatment groups. Only cows being bred a first or second time were included in the trial and equal numbers of animals receiving first and second services were assigned to each group.

Animals in the treatment groups (6-10 on each farm) were given IM injections of 10,000 IU HCG (Follutein®)[15] on day 10 to 12 after breeding by artificial insemination (AI). All animals on each farm, both control and treated, were inseminated by the same technician with frozen semen. Pregnancy diagnosis was by rectal palpation by the attending local veterinarian not earlier than 35 days after breeding. Heat observation was continued after breeding and the animals that showed signs of heat were rebred but not re-injected.

The conception rates and the intervals between services where conception did not occur were compared between the control and treatment groups.

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[15] Supplied by Dr. Stoner of the E.R. Squibb and Sons, Princeton, NJ 08540.

Statistical analysis

A 2-sample t-test (unpaired) was used to compare the estrous cycle lengths of each of the 2 treated groups of animals in part 1 with the controls. The post treatment cycle lengths for both groups were also compared with the controls. A similar comparison was made between the cycle lengths of the non-pregnant treated animals and the non-pregnant controls as well as between the conception rates of the pregnant treated and controls in part 2 of the study. The progesterone profiles for the treatment groups were compared with the progesterone profile for the controls using the split-plot method of analysis of variance (Gill and Hafs, 1971).

RESULTS

Part 1: Effect of HCG on CL function

Average estrous cycle lengths and distribution of cycle lengths for individual cows are presented on Table 2. The average cycle length for cows in group A, given HCG every 7 days beginning on day 9, 10 or 11 was longer ($P < .01$) than that for control cows (28.0 ± 1.7 vs. 19.9 ± 0.6 days, respectively). The average cycle length for group B cows, given HCG every 14 days beginning on day 9, 10 or 11 was also longer ($< .01$) than that for control cows (23.3 ± 0.7 vs. 19.9 ± 0.6 days, respectively). Group A cows had a longer ($P < .05$) average cycle length than cows in group B (28.0 ± 1.7 vs. 23.3 ± 0.7 days, respectively). Mean post treatment cycle length for both treatment groups (18.4 ± 0.9 days) did not differ from that for the controls ($P > .05$).

Figure 2 shows the average serum progesterone profile for cows in each treatment group. The serum progesterone levels became higher and remained high longer for the groups A and B animals compared to controls ($P < .01$). The major progesterone decline (i.e. luteolysis) occurred after day 23 for group A, day 18 for group B and day 17 for controls. Individual responses to HCG injections are presented on Table 3 and Figures 3 and 4. The first HCG injections increased the progesterone levels significantly in every animal in both treatment groups. Mean serum progesterone doubled following the first injection of HCG

TABLE 2

Distribution of cycle lengths of control and treated cows (Part 1)

Cycle lengths (Days)	Number of animals			
	Control	Treated		Post treatment cycle
		GP A	GP B	GPS A and B
14	-	-	-	1
16	-	-	-	1
17	-	-	-	2
18	2	-	-	-
19	3	-	-	1
20	2	-	1	2
21	2	-	-	1
23	-	1	1	1
24	1	-	3	-
25	-	-	1	-
26	-	1	-	-
27	-	2	-	-
30	-	1	-	-
35	-	1	-	
Total	10	6	6	9[c]
mean cycle length	[d] 19.9 ± 0.6[g]	[e] 28.0 ± 1.7	[f] 23.3 ± 0.7	[d] 18.4 ± 0.9

[c] Cycle length for the remaining 3 could not be determined.

[def] Values with different superscripts are different ($P < .05$).

[g] Standard error of the mean.

Figure 2. Average cycle lengths and serum progesterone levels for control cows and cows given HCG every 7 days (group A) or 14 days (group B) beginning on day 9, 10 or 11 of the estrous cycle

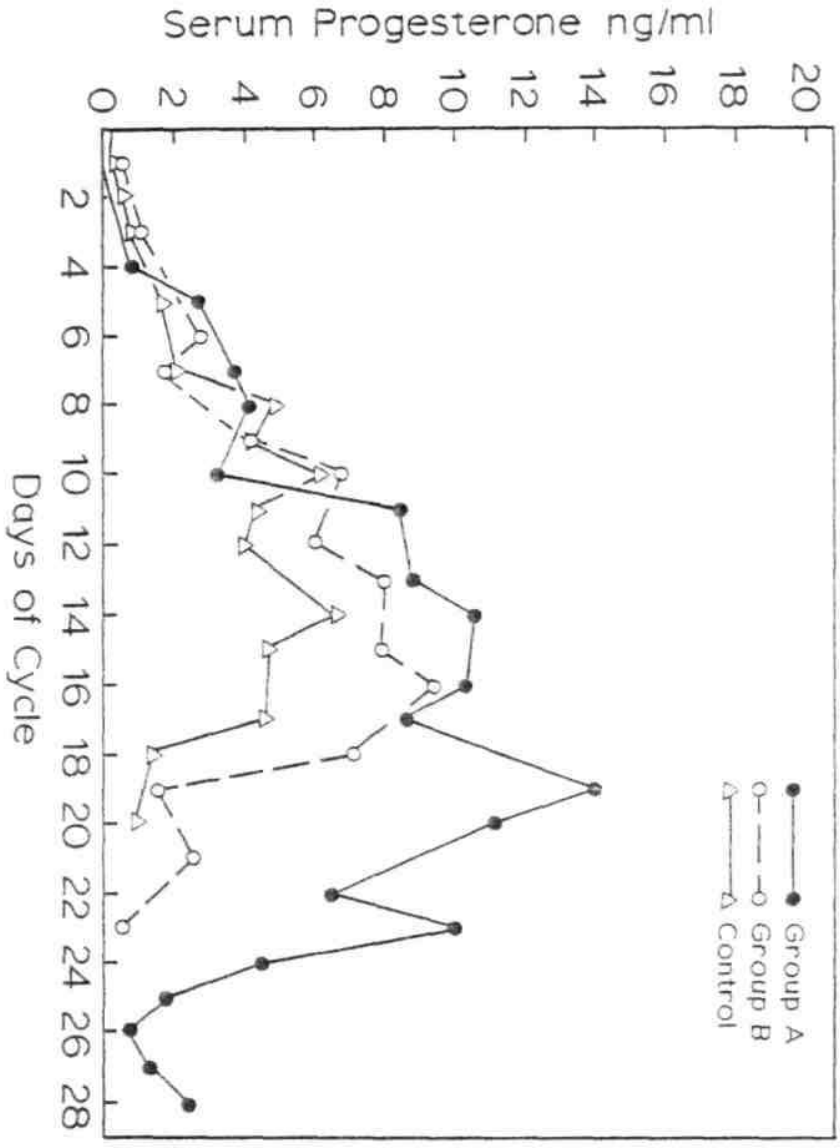

Serum Progesterone ng/ml
0
2
4
6
8
10
12
14
16
18
20
Days of Cycle
2 4 6 8 10 12 14 16 18 20 22 24 26 28
Group A
Group B
Control

TABLE 3

Effect of HCG injections on serum progesterone

GP A Cow	1st Injection (Day 9-10)[h] Serum Progesterone level Pre-Injection	Post-Injection	Change (%)	2nd Injection (Day 16-17) Serum Progesterone level Pre-Injection	Post-Injection	Change (%)
897	3.0	6.8	+126.7	4.3	1.3	-69.8
901	2.0	8.8	+340.0	10.3	16.0	+55.3
886	4.5	10.4	+131.1	13.5	20.1	+48.9
908	2.8	6.7	+139.3	11.3	7.2	-36.6
795	6.5	12.0	+84.6	12.5	14.0	+12.0
712	3.1	7.3	+135.5	6.8	5.8	-14.7
Mean	3.7±0.7	8.7±1	159.5±37.8	9.8±1.5	10.7±3	-4.9

GP A Cow	3rd Injection (Day 23-24) Serum Progesterone level Pre-Injection	Post-Injection	Change (%)	4th Injection (Day 30-31) Serum Progesterone level Pre-Injection	Post-Injection	Change (%)
897	0.9	1.6	+77.8	3.7	5.3	+43.2
901	0.9	7.9	+777.8	1.4	2.3	+64.3
886	7.2	0.4	-94.4	-	-[i]	-
908	0.6	0.8	+33.3	1.2	ND[i]	-
795	11.0	1.5	-86.4	1.4	1.9	+35.7
712	3.8	9.0	+136.8			
Mean	4.1±1.8	3.5±1.6	147.5	1.9±0.6	3.2±1.1	47.7±8.7

TABLE 3 - Continued

GP B Cow	1st Injection (Day 9-10) Serum Progesterone level Pre-Injection	1st Injection (Day 9-10) Serum Progesterone level Post-Injection	Change (%)	2nd Injection (Day 23-24) Serum Progesterone level Pre-Injection	2nd Injection (Day 23-24) Serum Progesterone level Post-Injection	Change (%)
551	3.4	4.2	+23.5	ND	0.7	-
891	4.1	6.7	+63.4	ND	ND	-
843	4.6	7.1	+54.3	ND	ND	-
758	1.4	11.0	+685.7	0.9	ND	-
884	3.4	6.9	+102.9	ND	0.5	
918	5.0	11.1	+122.0	ND	ND	-
Mean	3.7±0.5	7.8±1.1	175.3±105.2			

[h] Samples were collected every 3 days and injections of HCG varied relative to time of blood sampling.

[i] Non-detectable.

Figure 3. Effect of HCG injections on progesterone levels and estrous cycle lengths of individual cows given HCG every 7 days (group A) beginning on day 9, 10 or 11 of the estrous cycle

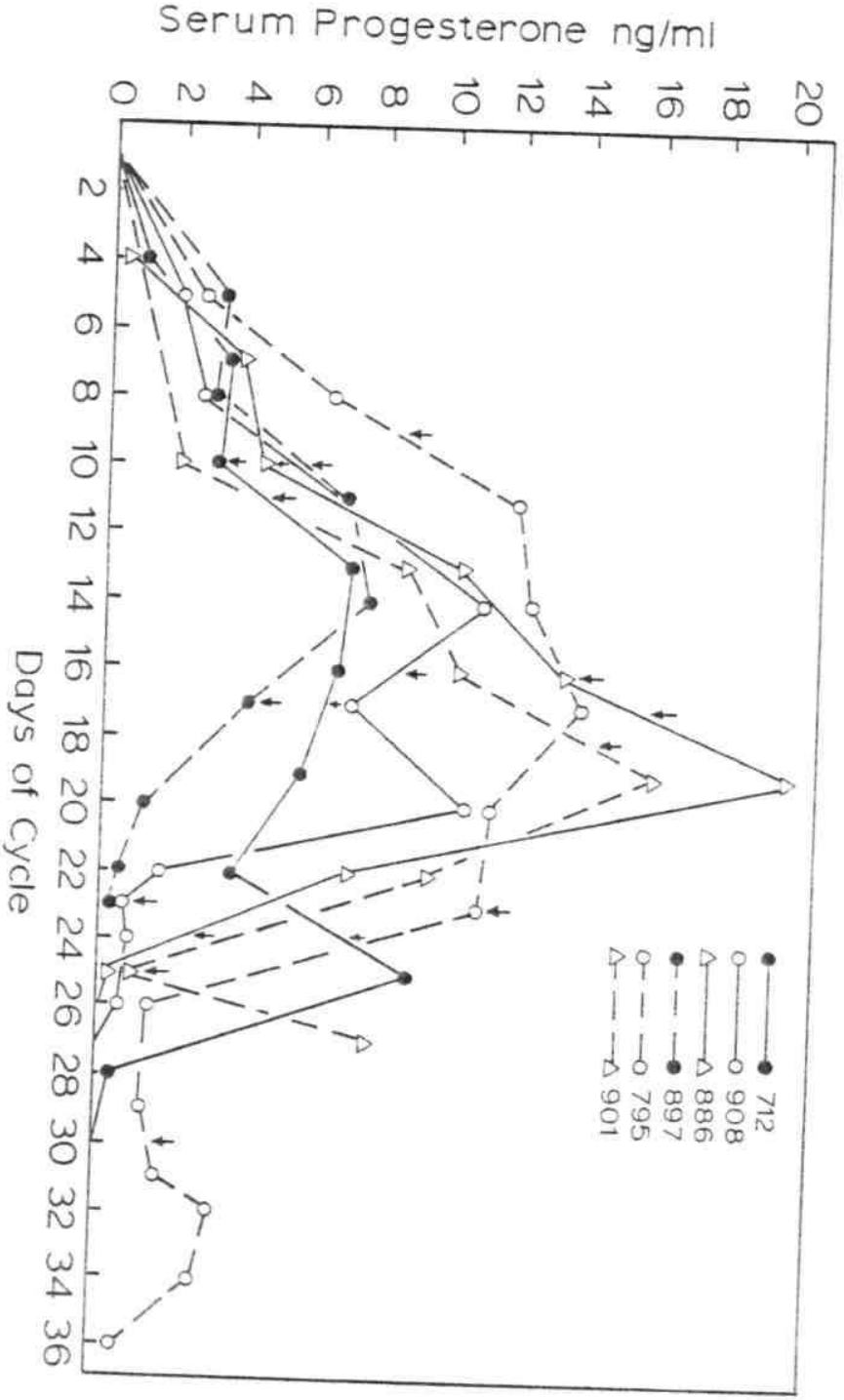
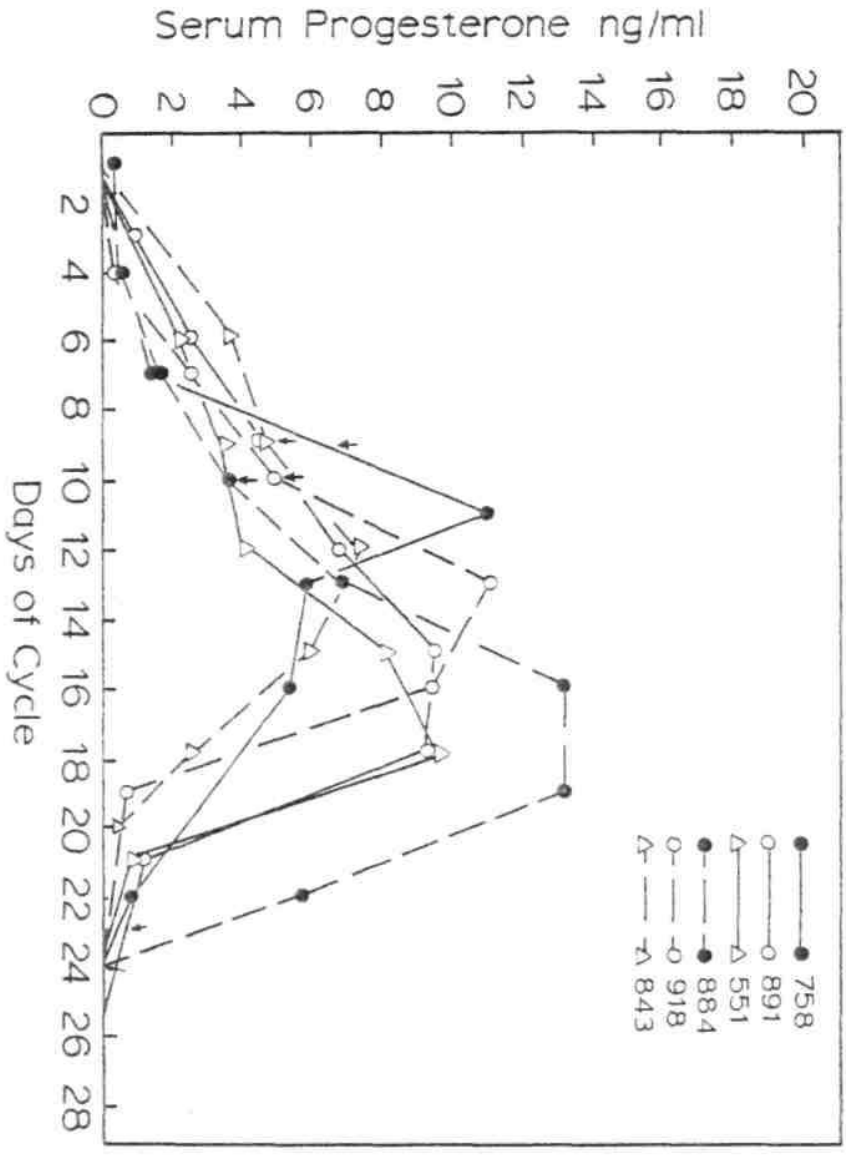

Serum Progesterone ng/ml
0
2
4
6
8
10
12
14
16
18
20
Days of Cycle
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
712
908
886
897
795
901

Figure 4. Effect of HCG injections on progesterone levels and estrous cycle lengths of individual cows given HCG every 14 day (group B) beginning on day 9, 10 or 11 of the estrous cycle


Serum Progesterone ng/ml
0
2
4
6
8
10
12
14
16
18
20
Days of Cycle
2 4 6 8 10 12 14 16 18 20 22 24 26 28
758
891
551
884
918
843

(Table 3). However, subsequent injections of HCG had variable effects on group A cows and the second and last injections in all group B cows had no effect on progesterone levels. Progesterone at the time of the second injection, 7 days after the first injection, in group A was high (9.8 ng/ml), but progesterone at the time of the second injection, 14 days after the first injection, in group B was low (< 1.0 ng/ml). Most group A animals had very low progesterone levels at the time of the third injection and some which had higher levels at the time of the fourth injection had probably gone into a new cycle.

At the time of the fourth HCG injections (group A cows) and second injections (group B cows) the CLs of most of the cows were either in their last phase of regression or had regressed beyond palpable limits. These regressing CLs did not respond to the HCG injections. One cow in group A (no. 886) showed obvious signs of heat shortly after the third injection. The other cows in group A did not show estrus at all or until after the fourth injection.

No precipitation bands were detected in Ouchterlony test for HCG antibodies.

Part 2: Fertility trial with HCG

Table 4 shows the number of animals bred and the percentage pregnant in both control and treatment groups for each farm. Mean conception rates for all farms were 63.4% for the control groups and 58.6% for the treatment groups. Among farms, pregnancy percentages varied from 20 to 84.6% in the control and 40 to 83.3% in the treated animals.

TABLE 4

Conception rates of heifers (one farm) and cows treated with HCG 10-12 days after breeding

Herd	Control			Treated		
	No. Bred	No. Pregnant	% Pregnant	No. Bred	No. Pregnant	% Pregnant
1	6	4	66.7	6	5	83.3
2	14	9	64.3	13	9	69.2
3	9	4	44.4	9	6	66.7
4	10	8	80.0	10	6	60.0
5	9	6	66.7	8	6	75.0
6	10	2	20.0	10	4	40.0
7	10	7	70.0	10	4	40.0
8	20	13	65.0	20	9	45.0
9	13	11	84.6	13	9	69.2
Total	101	64	63.4	99	58	58.6

Mean interval between services for the non-pregnant treated cows (29.3 ± 1.2 days) was longer ($P < .01$) than the mean interval for the non-pregnant control cows (24.1 ± 0.9 days). The distribution of cycle lengths (up to 40 days) of bred, but non-pregnant cows is shown on Table 5. While a majority of the non-pregnant animals in the control groups had cycle lengths of 22 days, the majority of those in the treatment groups had cycle lengths ranging from 22-25 days with much variation up to 40 days.

TABLE 5

Distribution of cycle lengths (not more than 40 days)

of bred but non-pregnant cows (Part 2)

Cycle Lengths (Days)	Number of Animals	
	Control	Treated
18	1	-
19	1	-
20	3	-
21	4	-
22	7	4
23	1	3
24	2	2
25	1	3
26	1	1
27	1	1
28	2	-
29	1	1
30	-	2
31	-	2
32	1	1
35	1	-
36	-	1
37	-	1
38	-	3
39	-	2
40	1	1
Total	28	28
Mean Cycle Length	24.1±0.9	29.3±1.2

DISCUSSION

The results of part 1 of this study indicate extension of the life span of the CL by HCG treatment in the 2 groups of animals. This is in agreement with previous reports (Katzman et al, 1931; Hisaw, 1944; Bryans, 1951; Wiltbank et al, 1961; Seguin et al, 1977). A difference of 4.7 days in the duration of CL life span extension between the groups A and B cows was observed. The reason for this might be that the weekly administration of HCG made HCG available to the CL over a longer period than when given every 14 days. Wiltbank et al 1961, extended the estrous cycle of heifers by 14.7 days with a daily injection of 1000 IU HCG from day 15 to the 26th day. Extension of the estrous cycle by 3.4 days with 2 injections of HCG 14 days apart in cows whereas a single injection caused a 7 day extension in heifers (Seguin et al, 1977), suggests a difference in response of the CLs of cows and heifers to HCG treatment. A good indication of the secretory activity and functional life of the bovine CL is the progesterone profile since serum progesterone levels have been shown to fall abruptly after CL enucleation (McCracken, 1963a), and rise and fall with the development and regression of the CL during the estrous cycle (McCracken, 1963b; Plotka et al, 1966). For this reason, the serum progesterone profile plus the palpation data were used to determine the estrous cycle lengths for all the cows in this experiment.

The mechanism by which HCG prolongs the life span of the CL is not known. A review of the mechanism of steroid production by the luteal cells indicates that following the ovulatory surge of LH, this hormone enters the follicular fluid (McNatty et al, 1975) and the plasma membrane LH receptors on the granulosa cells (Channing and Kammerman, 1974) become saturated leading to morphological and functional luteinization of the granulosa cells. Similarly, HCG which also has receptors on the granulosa cells (Hichens et al, 1974) may saturate these receptors when administered. In addition, it has been shown in in vitro studies that HCG competes with LH for specific saturable binding sites on bovine CL plasma membrane (Gospodarowicz, 1973). A possible explanation for prolongation of CL life span by HCG may be that HCG, by occupying receptor sites on the plasma membrane of the granulosa cells, masked the receptors for the uterine luteolysin and prevented luteolysis. Seguin et al (1977) suggested that HCG may override the uterine luteolytic message either by protecting the CL directly or by inhibiting luteolysin release at the uterine level. Denamur (1968) postulated that estrogen produced from new follicles induced by LH or HCG administration (Schomberg et al, 1967) neutralized the uterine luteolytic effect resulting in a prolonged CL maintenance.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been shown to be the natural luteolysin secreted by the uterus in the ewe (Goding, 1974) and substantial evidence exists to show that exogenously administered

$PGF_{2\alpha}$ acts as a luteolysin by causing cessation of progesterone secretion and structural regression of the CL in several species (Pharris and Wyngarden, 1969 in rat; Blatchely and Donovan, 1969 in guinea pig; Pharris, 1970 in rabbit; Gutknecht et al, 1971 in hamster; McCracken et al, 1972 in ewe; Rowson et al, 1974 in cow). Also, a suggestion that the CL is regulated by an interplay between a pituitary luteotrophin and a uterine luteolysin has been advanced (Short, 1967). Henderson and McNatty (1975, 1977) hypothesized that the saturation and binding of the luteal cell receptors by LH protects a young CL since it is refractory to $PGF_{2\alpha}$ for the first 4 days after its formation (i.e. after ovulation) in the cow (Rowson et al, 1972; Hendricks et al, 1974), horse (Allen and Rowson, 1973), sheep (Hearnshaw et al, 1973) and for the first 12 to 14 days in the pig (Moeljono et al, 1976). As the receptor for $PGF_{2\alpha}$ is also located in the plasma membrane (Powell et al, 1974, 1975), it is possible too that the receptor-bound HCG antagonized the uptake of $PGF_{2\alpha}$ by the luteal cells. Behrman et al (1971) reported that LH antagonized the luteolytic action of $PGF_{2\alpha}$ in the rat.

As the HCG begins to vacate the receptors by dissociation or by neutralization by a possible anti-HCG formed, the CL becomes susceptible to the luteolytic actions of the luteolysin. It is well established that the dissociation of LH from its receptors is a slow process taking several days (Lee and Ryan, 1971: Catt

et al, 1972; Channing and Kammerman, 1973; Haour and Saxena, 1974). This may also be true of HCG and this might have contributed to the extension of the cycle observed in this study.

Most of the cows in this study failed to show signs of estrus following HCG treatment even though they lacked palpable CLs. This observation is similar to that made by Snook et al (1971) in ewes that lacked functional CLs, but did not show signs of heat for 33 days following immunization with HCG. These workers attributed this failure of the ewes to show heat signs to increased serum LH resulting from the immunization of these ewes with HCG (Cole et al, 1965). Although the cows in this study did not show signs of estrus, the progesterone profile indicated that these animals actually came in heat in spite of treatment. Also, it was observed that at the time of the third or fourth (in some cows) HCG injections the CLs had started to regress. These regressing CLs could not be revived by the injections. This finding appears to support the observed bleeding in women despite continued HCG treatment (Brown and Bradbury, 1947).

The double diffusion plate technique of Ouchterlony (1958) test for antibodies failed to reveal detectable antibodies against HCG. This, however, would not rule out the presence of anti-HCG in these samples; perhaps the anti-HCG titer was too low to be detected by this method. Snook et al (1971a) had observed that

anti-HCG titers in ewes 16 days after the first series of a three-series weekly immunization injections of HCG, as measured by a RIA procedure utilizing an anti-serum to bovine LH (Snook et al, 1971b), were low. The anti-HCG titers of these ewes increased following a second series of injections.

Thus, the cause of failure of a continued CL maintenance by the repeated HCG injections could not be determined in this study. It is, however, possible that anti-HCG was formed and this neutralized the HCG thereby making the CL readily susceptible to the lytic effect of $PGF_{2\alpha}$. Serum from HCG-treated rabbits neutralized the ovulation-inducing activity of HCG (Reel et al, 1976). Furthermore, Thompson's (1941) conclusion that ovulatory refractoriness in rabbit was due to an immune reaction to an exogenous heterologous hormone (e.g. HCG) has been supported by several studies (Leathem, 1947; Adams, 1953; Adams, 1961; Lin and Bailey, 1965; Land and McLaren, 1967; Mauer et al, 1968; Greenwald, 1970). Another possibility for the repeated HCG injections not continuously preventing CL regression is that 7 days was too far apart. As the HCG is shed from the receptors and blood concentrations are low, regression may begin.

In the present trial, the HCG treatment had no effect on the subsequent estrous cycles. This supports the previous reports of normal subsequent cycles following HCG treatment in women (Brown and Bradbury, 1947) and in the cow (Donaldson and Hansel, 1965).

One interesting observation in this experiment was the sharp increase in serum progesterone measured 1 to 3 days following the first HCG injection in all the cows. All the cows but 1 had over 50% increase. This finding is consistent with those reported by several other workers in different species (Neil and Krobil, 1972; Surve et al, 1973) in monkeys (Strott et al, 1969; Hanson et al, 1971) in women and (Seguin et al, 1977) in cow. However, it is doubtful if the increased serum progesterone has any practical value judging from the results obtained from part 2 of this study. The results of both experiments confirm the luteotropic properties of HCG. If a normal CL in the cow is misdiagnosed as cystic and the cow is treated with HCG, a delayed return to estrus is likely to result.

The results of the fertility trial with HCG indicate that HCG given to first or second service dairy cows and heifers 10-12 days after breeding did not improve conception rate. This finding is similar to that of Wiltbank et al (1961), who found no significant increase in conception rate following a daily administration of 1000 IU HCG to heifers from the 15th day to 35th day after breeding. When administered 96 hours after estrus, HCG also failed to improve pregnancy rate in heifers (Morris et al, 1976). However, in contrast to these findings, injections of 2000 or 1500 IU HCG at insemination to synchronized and non-synchronized heifers increased pregnancy percentage (Brown et al, 1973; Wagner et al, 1973). Hansel et al (1960, 1976) did not improve pregnancy rate with HCG given to cows at insemination.

The rationale behind the HCG treatment 10-12 days post breeding was to delay estrus by prolonging CL function and thereby giving the expected embryo a better (i.e. longer) than normal chance to become established in the uterus. The HCG treatment in this study delayed estrus of the non-pregnant animals as the mean interval between services for the treated groups was significantly longer ($P < .01$) than the interval for the controls. This result is consistent with that of Seguin et al (1977), who extended CL function with 10,000 IU HCG given on day 10 or 11 of the estrous cycle. The reason for the failure of a prolonged CL function to improve conception rate could not be determined from this study. This failure implies that the use of HCG in this way has no practical benefits and thus can not be recommended.

SUMMARY AND CONCLUSIONS

A study of the effect of HCG on bovine corpus luteum function and its subsequent effect on fertility was undertaken in 2 parts. In part 1, all cows in 2 treatment groups (6 cows per group) were given IM injections of 10,000 IU HCG beginning on day 9, 10 or 11 of the estrous cycle; in group A injections were repeated every 7 days and in group B every 14 days. Group A animals received a total of 4 injections each while group B animals received 2 each. Control cows were given isotonic saline on day 9, 10 or 11. The estrous cycle lengths of groups A and B cows were prolonged by an average of 8.1 days and 3.4 days, respectively. Progesterone levels after the first HCG injections increased by over 50% in all but 1 cow in both groups. One cow had a 23.5% increase in group B. Corpus luteum regression occurred in spite of continued injections of HCG. At the time of the third and fourth injections in group A and the second injections in group B, the CLs of most of the animals had regressed to non-palpable limits and some of the animals had entered the next cycle. Mean post treatment cycle length was not different from that of controls. Antibodies against HCG were not detected in serum samples collected 2 weeks after the last HCG treatment.

In part 2 (fertility trial) of this study the mean conceptio rate of dairy cows treated with HCG (10,000 IU) 10-12 days post breeding was similar to that of controls. However, HCG treatment increased the average interservice interval in the non-pregnant animals (24.1 vs. 29.3 for control and treated, respectively

In conclusion, periodic injections of HCG beginning on day 9, 10 or 11 of the estrous cycle prolonged the life span of the CL, increased its secretory activity and delayed the next estrus. This, as well as the prolonged interservice interval in bred but non-pregnant cows, confirm the luteotrophic properties of HCG. The schedule of treatment in part 1 could only sustain the CL for a limited period and a regressing CL could not be revived by HCG injection. Human chorionic gonadotropin treatment of a cow with a normal CL will result in a delayed estrus. The subsequent cycles of the animals were not affected by the HCG treatment. Prolongation of the estrous cycle with HCG did not improve pregnancy rate in dairy cattle.

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