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Two experiments were conducted to examine the effects of passive immunization of pregnant mares with a monoclonal antibody [MAb, 518B7, antibovine βLH which crossreacts with equine chorionic gonadotropin (eCG)] on subsequent reproductive characteristics and changes in plasma concentrations of hormones. In Exp.1 control mares (C, n=3) were not treated. Another group (PGF, n=2) received prostaglandin $F_2\alpha$ ($PGF_2\alpha$, 5 mg, i.m.) on d 45 and every 12 h until abortion. A third group (PGF/MAb, n=4) received $PGF_2\alpha$ as in group PGF plus MAb (35 mg, i.v.) on d 45 and 49. Because there were no significant differences in plasma concentrations of eCG and progesterone between groups PGF and PGF/MAb, data were combined. Treatment of mares with PGF or PGF and MAb caused a transient plateau or slight decrease in plasma eCG concentrations for 2 to 24 h compared with that of controls. However, on d 46, 47, 48 and 51 eCG concentrations were lower in treated mares ($P < .05$). Plasma progesterone in treated mares decreased to < 1 ng/ml within 24 h. Abortion occurred in all treated mares (mean ± SE, 5.8 ± 1.0 d) but only four of six mares exhibited post-treatment estrus (d 2, 4, 4 and 28) with a duration of estrus of 4.8 ± 1.0 d (mean ± SE); two mares

exhibited estrus before abortion. The interval from treatment to first ovulation was 8.3 ± 1.0 d. There was a tendency for treated mares (aborted) to have fewer luteal structures (secondary corpora lutea or luteinized follicles) than in pregnant control mares (5.2 vs 8.0 structures, respectively; P = .13). Proportion of secondary corpora lutea (CL) (66%) to luteinized follicles (33%) was similar between groups. None of the treated mares that exhibited estrus with ovulation subsequent to abortion conceived at breeding; eCG remained elevated until end of the experiment on d 100.

In Exp.2 repeated treatment with MAb (MAb, n=3) on d 35 (25 mg, i.v.), 36 (35 mg), 37 (45 mg) and 38 (55 mg) appeared to delay eCG production in treated vs control (C, n=3) mares during this time. However, mean concentrations of eCG were significantly lower ($P < .05$) on d 39 only in MAb treated mares (.05 IU/ml) but increased after d 40 and were similar to that of controls (13.7 IU/ml). Plasma progesterone was lower ($P < .05$) in treated mares on d 36 only (4.8 vs 7.8 ng/ml in controls). Thereafter, progesterone increased in mares of both groups and did not differ. Treated mares tended to have a higher proportion of luteinized follicles (70%), which occurred later in gestation (mean = d 71); control mares had more ovulatory follicles (78%, mean = d 58).

In both experiments treatment with MAb, at the times and dosages used, had only a short-term, transient effect on eCG, whether mares had aborted (Exp.1) or remained pregnant (Exp.2). The significant reduction in plasma progesterone in pregnant MAb-treated mares (Exp.2) on d 36,

when eCG is usually low or undetectable, was unexpected. This may indicate a necessity for continued tropic stimulation of the primary corpus luteum by luteinizing hormone before appearance of eCG.

Reproductive Characteristics of Pregnant Mares Passively Immunized Against Equine Chorionic Gonadotropin

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INTRODUCTION

Endometrial cups are temporary glands that form in the uterus of the pregnant mare and secrete equine chorionic gonadotropin (eCG) from d 35 to 120 of gestation (Cole and Goss, 1943; Clegg et al., 1954; Allen and Moor, 1972). In the event of pregnancy failure after their establishment function of endometrial cups is retained and they continue to secrete eCG to d 120 (Allen, 1975c). Consequently, mares in which pregnancy fails while endometrial cups are present often fail to resume normal cyclic activity until the endometrial cups have degenerated and eCG is no longer produced (Douglas et al., 1974; Allen, 1978; Rathwell et al., 1987). Absence of normal estrous cycles following abortion reduces the opportunity for rebreeding during the same breeding season.

Two experiments were conducted with the following objectives: 1) to determine if passive immunization of pregnant and aborted mares with a monoclonal antibody against the beta subunit of eCG would effectively reduce circulating plasma eCG concentration; 2) to determine effects of passive immunization on subsequent reproductive characteristics such as secondary ovulations, follicular luteinizations and function of corpora lutea reflected by changes in concentrations of plasma progesterone and 3) to determine if passive immunization would

facilitate resumption of normal estrous cycles following abortion. In conjunction with the study, a competitive, semiquantitative enzyme linked immunosorbent assay (ELISA) was developed to measure endogenous eCG concentrations.

LITERATURE REVIEW

Estrous Cycle and Initiation of Pregnancy

The pregnant mare has provided researchers with the opportunity to study a reproductive system with a number of unique physiological features. Development of secondary follicles, accessory ovulations follicular luteinizations and production of equine chorionic gonadotropin (eCG) are features of equine pregnancy not found in other farm species. A thorough review of reproductive anatomy and physiology of the mare has been provided by numerous textbooks (Ginther, 1979; Neely et al., 1983; Rossdale and Ricketts, 1980).

Horses are seasonally polyestrous breeders, with a gestation period of 335 to 340 days. Photoperiod appears to be the major factor influencing seasonality. Increasing photoperiod inhibits the rhythmic release of melatonin from the pineal body. Seasonal reduction in melatonin in turn stimulates rhythmic release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary which act upon the ovary to initiate estrous cycles (Sharp, 1981).

Estrous cycles average 21 to 23 d. Length of estrus is variable but is generally of 4 to 7 d in duration. Ovulation occurs 12 to 24 h before the end of estrus. In nonpregnant mares, estrus is followed by a 14 d diestrous phase in which the corpus luteum (CL) is functional (Ginther, 1979). The fertilized ova enter the uterus within 5 to 6 d. Transuterine migration of the embryo continues until d 17 (Ginther,

1983) Extensive mobility of the early conceptus is thought to initiate physiological maternal recognition of pregnancy. Movement of the embryonic vesicle at the critical time period (15 to 16 d) suppresses endometrial synthesis and(or) release of the active luteolysin prostaglandin $F_2\alpha$ ($PGF_2\alpha$) (Sharp, 1980). Hence, luteostasis is achieved and maintenance of pregnancy is temporarily assured. In the absence of a viable embryo, however, $PGF_2\alpha$ release results in luteolysis and return to estrus. Therefore, the presence of the embryo rescues the primary CL and ensures continued secretion of progesterone for maintenance of pregnancy. In early pregnancy, progesterone is essential for synthesis and secretion of embryotrophic proteins by the uterus and is associated with an increased thickening and tone of the uterus and cervix, all of which contribute to normal pregnancy maintenance (Ginther, 1979).

Endocrinology of Pregnancy

Concentrations of serum progesterone in the pregnant mare reflect both ovarian and placental activity throughout gestation and have been studied extensively by use of competitive binding assay (Allen and Hadley, 1974; Holtan et al., 1975) and radioimmunoassay (Burns and Fleeger, 1975; Ganjam et al., 1975). Studies by Holtan et al. (1975) showed that plasma progesterone concentrations increase from ovulation to d 10 and reach levels of approximately 10 ng/ml. A slight decline in plasma concentrations of progesterone occur between d 12 and 30. Presumably, this decline in secretory activity of the primary CL occurs about the time that luteolysis occurs in the normal cycling animal.

However, in the event of physiological recognition of pregnancy, the uterine luteolytic mechanism is inhibited and the primary CL is maintained. A pronounced secondary rise in progesterone occurs about d 30 to 45 in response to development of accessory CL originating from waves of follicular growth between days 40 and 150 of pregnancy (Cole and Hart, 1930; Squires and Ginther, 1975). Accessory ovulations and luteinization may give rise to none or as many as 30 accessory secondary CL by d 120 (Amoroso et al., 1948; Allen, 1984). Concurrent with the development of accessory CL, progesterone concentrations achieve a plateau that generally extends from 60 to 120 d at which time average concentrations of plasma progesterone are approximately 15 ng/ml. The CL-dominated phase of pregnancy ends between d 160 to 180 (Squires and Ginther, 1975) when all of the CL, both primary and secondary, regress together and cease to function leaving small inactive ovaries for the remainder of gestation. At this time, plasma progesterone levels decline (1 to 2 ng/ml) and are maintained until late gestation near 300 d.

As early as 1956 it was known that placental tissues contained significant quantities of progesterone (Short, 1956). Subsequently, the secretion of placental progesterone was shown to be sufficient to maintain pregnancy from about d 100 postovulation. Holtan et al. (1979) reported that mares ovariectomized on d 140 remained pregnant whereas only half of those ovariectomized as early as 60 to 70 d were able to maintain pregnancy without ovarian progesterone. Therefore, production

of placental progesterone likely begins before regression of corpora lutea and continues throughout gestation despite luteolysis of all corpora lutea about d 160 to 180.

Limited studies have reported serum concentrations of LH and FSH in the pregnant mare. Absolute levels of LH begin to increase a few days before the onset of estrus. Peak serum concentrations of LH are achieved near ovulation or shortly thereafter. Plasma concentrations then decrease during the next 4 or 5 d to < 2 ng/ml and remain low to d 32 (Ginther, 1979). Because of cross reactivity, current assay methods are not able to differentiate LH from eCG which appears between 37 and 150 d of gestation. However, after disappearance of eCG, systemic LH concentrations remain at baseline levels. Therefore, after the ovulatory surge, LH levels likely remain low (Nett et al., 1975) throughout gestation.

Irvine and Evans (1976), measured FSH levels during the estrous cycle and in pregnant mares to d 78. Surges of mean plasma FSH concentrations were recorded between 20 and 120 ng/ml at 10 to 11 d intervals in both cyclic and early pregnant mares. Urwin and Allen (1982) reported similar hormone concentrations but were unable to establish a uniform 10 d pattern of release. Regardless of cyclic release, average FSH concentrations in the early pregnant mare are comparable to those found on the corresponding days of diestrus in the cyclic mare. Concentrations during the first 60 d are relatively high followed by a decrease to baseline concentrations sometime before d 150. Thereafter, eFSH concentrations remain low until a final surge of this gonadotropin takes place before parturition.

The remaining gonadotropin of interest is eCG, formerly referred to as pregnant mare serum gonadotropin (PMSG). Cole and Hart (1930) first reported the presence of a gonadotropic substance in the serum of mares in early pregnancy. Their initial work demonstrated that serum of mares injected into immature rats stimulated growth of the reproductive system. The hormone was named pregnant mare serum gonadotropin (PMSG). Early reports of eCG included a characterization of circulating levels in pregnancy and a proposal for its use in diagnosis of pregnancy. Early researchers presumed it to be of pituitary origin.

Early assays for eCG were bioassays based on changes in ovarian and uterine weight of immature rats and mice (Cole and Erway, 1941; Schmidt-Elmendorff et al., 1962). The first immunoassay was described by Wide and Wide (1963), in which a rabbit anti-eCG serum was absorbed with nonpregnant mare serum in a quantitative hemagglutination-inhibition system. This method of measurement was widely accepted until W.R. Allen (1969b) developed a turkey anti-PMSG serum specific for use in an unabsorbed hemagglutination-inhibition assay. Results from use of this assay (Allen, 1969c) were comparable with those obtained by bioassay (Holtan et al., 1975). Nett et al. (1975) developed a radioimmunoassay for LH with high eCG cross reactivity. This assay was reported to be considerably more sensitive than previous hemagglutination-inhibition assays. Investigations on circulating concentrations of eCG have been reviewed (Catchpole and Lyons, 1934; Day and Rowlands, 1940; Allen, 1969c). There is general agreement among researchers that eCG is first detectable by d 35 to 40 of pregnancy. Average maximum plasma concentrations (100 to 150 IU/ml) are observed at 55 to 65 d. The

hormone concentration then slowly declines to nondetectable levels by d 120 to 150. Maximum plasma concentration varies tremendously among individual mares, jenny donkeys and hybrid crosses. Reports range from 11 IU/ml (Allen, 1969c) to 600 IU/ml (Spincemaille, 1975) in different individual mares, 30 to 60 IU/ml in jenny donkeys (Day and Rowlands, 1940; Allen, 1969c), 12 to 22 IU/ml in mares carrying mule conceptuses and 180 to 250 IU/ml in jennys carrying horse conceptuses (Allen, 1975c).

Biochemistry of Equine Chorionic Gonadotropin

Biochemically, eCG has been characterized as a glycoprotein hormone with a total molecular weight of 53,000 daltons (Gospodarowicz, 1972). It is distinguished by an unusually high carbohydrate content which approaches 45% compared with the 25% carbohydrate content of eFSH and eLH. In addition to neutral sugars and hexosamines, eCG possesses 13.5% sialic acid (Papkoff, 1974). Sialic acid is essential to all gonadotropins for biological activity and is believed to prolong the half-life of glycoprotein hormones by inhibiting their uptake and degradation in the liver (Morell et al., 1971). The high sialic acid content of eCG then might account for its relatively long half-life. The half-life of eCG in geldings and hysterectomized mares is reported to be 6 d (Cole et al., 1967) and 24 to 26 d in rabbits (Catchpole et al., 1935). The observation that eCG has the same half-life in both geldings and hysterectomized mares suggests that the ovaries do not play a major role in eCG metabolism. Early studies suggested that eCG was

fully metabolized in the horse and not excreted in urine (Catchpole et al., 1935). However, Cole et al. (1967) estimated that as much as one-seventh of the total circulating eCG was excreted in urine.

Chemical investigations have shown that eCG, like other glycoprotein hormones, consists of two chemically dissimilar, covalently linked subunits, alpha and beta. The alpha subunit, is structurally similar among the various hormones (FSH, LH, eCG, TSH). The beta subunit, is specific to each and determines the biological activity of the respective hormones (Pierce and Parsons, 1981). Each subunit of eCG represents a single polypeptide chain. The alpha subunit is the smaller of the two eCG subunits, approximately 17,000 daltons, whereas the beta subunit ranges between 38,000 and 44,000 daltons (Christakos and Bahn, 1979; Moore and Ward, 1980). Amino acid composition of the two chains are typical of glycoprotein hormones, and strongly resemble LH because the content of cystine and proline is high and the content of histidine, methionine and tyrosine is relatively low (Papkoff, 1981).

As in other species, the alpha subunits of all equine gonadotropins (eFSH, eLH, eCG) are similar. Moore and Ward (1980) determined the amino acid sequence of eCG alpha and found it to be nearly identical to eFSH alpha. The eCG beta subunit shares no homology with eFSH beta but exhibits nearly 85% homology to eLH beta which may explain the display of luteotropic activity of eCG in the mare (Moore and Ward, 1980). This does not, however, explain the FSH-like activity of eCG in other species. Dissociated subunits of eCG are inactive with respect to LH and FSH activity. However, upon recombination, both LH (23-32%) and FSH (28-44%) activity is restored (Papkoff, 1974; Moore and

Ward, 1980). Different combinations of an alpha subunit of one hormone with a beta subunit of another result in biological activity consistent with the hormone from which the beta subunit was obtained (Sherwood and McShan, 1977).

Purified eCG was isolated from pregnant mare low and high titer serum (Aggarwal et al., 1980; Papkoff et al., 1978a), endometrial cup tissue (Papkoff et al., 1978b) and trophoblast cell culture medium (Aggarwal et al., 1980). The properties of eCG, both chemical and biological (LH and FSH activity), often vary depending on the source from which they are derived; eCG isolated from low titer serum and endometrial cup tissue possesses far less FSH and LH activity than eCG from high titer serum (Papkoff et al., 1978a). Additionally, eCG from high titer serum has a higher carbohydrate content than that of endometrial tissue (Aggarwal et al., 1980). In this regard, Manning et al. (1987) reported that removal of the sialic acid component from the eCG molecule increased its LH activity. They further proposed that the variability in biological activity and concentrations of eCG between individual mares might be attributed to the degree of glycosylation of the eCG molecule in vivo. Carbohydrate content of the molecule may alter the clearance rates of the hormone therefore alter its concentration without affecting its production. This might explain some of the biological variation observed in individual mares with regard to number and relative activity of accessory ovulations and luteinizations during pregnancy. Others argue that the differences in biological activity is attributed to the heterogeneity of the eCG molecule (Moore and Ward, 1980).

Origin and Function of Endometrial Cups

Although early studies characterized circulatory levels of eCG, until recently, little was known about its source of secretion. Early researchers believed eCG was maternal in origin, a product of the pituitary gland. Although J.C. Ewart (1897) first observed the presence of temporary endometrial growths and others alluded to their presence and function (Cole and Hart, 1930), Catchpole and Lyons (1934) were the first to propose that the gonadotropin may be produced by the placental unit. Forty years later, Allen (1972) proposed that eCG originated from the uterine endometrial cups. More notably, it was proposed that the cups were of fetal and not maternal origin. Allen's first paper in a series (1972) provided morphological and biochemical evidence that endometrial cups, originated from a transitory circumferential thickening of the fetal chorion called the allantochorionic girdle. His hypothesis was confirmed in a two-part experiment in which tissue from the allantoic girdle, normal allantochorion, fetal skin and maternal endometrium were cultured and then assayed for eCG. Only the allantochorionic girdle cells produced eCG. Levels of 400 IU/ml were reached within 30 d. These concentrations far exceeded the maximum achieved in the blood serum of pregnant mares. In addition, levels of 5 to 30 IU/ml were still present after 180 d whereas eCG in vivo is detectable for only 80 d. In a second experiment, tissue samples of trophoblastic cells were grafted from the allantochorionic girdle to the endometrium of a recently aborted mare and to the testis of a yearling

colt. As a result of the graft, the nongravid uterine endometrium developed endometrial cups and the testis graft site developed small groups of cells displaying the same morphological characteristics observed in the endometrial cup cells. The results of these studies confirmed that equine trophoblast cells recovered from the allantochorionic girdle at a specific stage of gestation produced eCG in vitro and only allantochorionic girdle cells had the potential to produce eCG whether in vitro or transplanted to different host sites in vivo. Hence, endometrial cups were composed of fetal cells originating from the allantochorionic girdle and not of maternal origin as hypothesized previously.

Allen et al. (1973) further described the origin of the endometrial cups from development of the chorionic girdle to the establishment of mature endometrial cup tissue. They reported that at approximately 37 d of pregnancy, specialized fetal trophoblastic cells from the allantochorionic girdle interdigitate with the endometrial epithelium forming a temporary attachment of the conceptus to the endometrium. The attachment soon progresses to an invasive phase. Pseudopodia form on the apical surface of the trophoblastic cells and invade and phagocytize the adjacent endometrial epithelial cells. A migratory phase follows in which the trophoblastic cells intrude further through the basil lamina into the endometrial stroma where they become sessile and hypertrophy, differentiating into mature cup cells. The morphology of the girdle cells reflects their invasive, migratory and phagocytic properties (Hamilton et al., 1973; Hernandez et al., 1975; Yamauchi, 1975). They are amoeboid in appearance and contain a

large nucleus and nucleolus indicative of nuclear directed cell activities. Specialized extracellular matrix material helps to bind fetal and maternal surfaces together and may also aid in the digestion of epithelial cell debris.

The term "endometrial cup" appropriately describes the gross appearance of these ulcer like endometrial growths. Mature cups are raised above the surface of the endometrium and possess a depressed center surface. They develop into various shapes: some round, others oblong or band-like. The depressed center of the cup holds a pocket of proteinaceous substance secreted by uterine glands. Presumably, eCG produced by the endometrial cup cells reaches the general circulation through the highly developed lymphatic area at the base of the cup. However, high concentrations are found in the cup center. Although the allantochorion lies over the endometrial cups it is not attached to the cup tissue. Such attachment between the chorionic girdle and the endometrium terminates after the invasion of the girdle cells into the endometrium. Beyond the periphery of the cups the fetal placenta is attached via interdigitation between microvilli of the trophoblast and apposing endometrial epithelium. Placentation is completed by 150 d (Samuel et al., 1976). Cups are arranged in a ring in the caudal portion of the gravid uterine horn. Orientation of the ring is dependent upon the orientation of the conceptus because the ring of cups develops wherever the chorionic girdle of the conceptus contacts the uterine endometrium. Sloughing of the cups occurs between 70 to 100 d and is completed by about 130 to 150 d (Clegg et al., 1954). Before sloughing, the cups become pale and necrotic in appearance. Honey-like

material consisting of necrotic cell debris and uterine-gland secretion accumulates on the surface of the degenerating cups. Eventually, the entire cup detaches to either lie free in the uterine lumen between the placenta and endometrium or become enclosed in folds of allantochorion. The latter, appropriately termed allantochorionic pouches, were observed from 130 to 300 d of pregnancy (Clegg et al., 1954). Their contents probably are retained or absorbed into the fetal circulation.

Results of light and electron microscope work reported by Hamilton et al. (1973) includes the description of the principle cell type found in endometrial cups. Cup cells typically are large, binucleated and epithelial in nature. Large nucleoli and excessive amounts of rough endoplasmic reticulum suggest that their function is to synthesize and secrete proteins. However, the mechanism by which they secrete their product remains unknown for there is an absence of membrane bound secretory granules. Conversely, there is evidence that some glycoprotein-hormone synthesizing cells do not package their secretory products into granules. Such is the case with Immunoglobulin G producing plasma cells (Farquhar, 1970). From histological observations the first concrete evidence became available for a maternal cell mediated regulatory mechanism that presumably initiates the sloughing of cup tissue at approximately 70 d. Allen et al. (1975c) reported that throughout the 80 to 100 d life span of cup cells there is a pronounced accumulation of lymphocytes, plasma cells and eosinophils in the endometrial stroma around the periphery of the cup tissue. Coincidentally, at about d 70, as the cup cells degenerate, leukocytes actively invade and destroy cup cells. Allen suggested that this

reaction represents a maternal cell mediated immune response to foreign antigens expressed by the fetal cup cells. This hypothesis was supported by the finding that 94% of normal intraspecies horse pregnancies studied produced strong cytotoxic antibody responses to paternal histocompatibility antigens (Kydd et al., 1982). Additionally, Spincemaille (1975) observed extremely high concentrations of eCG in heterosexual chimeric twin mares mated with their co-twin brothers. Unusually high eCG concentrations were detected at 220 to 260 d as compared with 90 to 113 d in normal pregnant mares. These data suggest that normal recognition of fetal paternal antigens expressed by endometrial cups did occur or was delayed because of the immunological tolerance demonstrated by the chimeric twins. However, the validity of this hypothesis cannot be determined as Kydd et al. (1982) also reported that the maternal antibody response from interspecies and extraspecies pregnancies in jenny donkeys were observed less frequently and more irregular than in horses. The donkey's failure to produce a cytotoxic response may indicate an alternative stimulus for antibody production other than the endometrial cups or perhaps the subtle differences in placentation between species accounts for the irregularity of response. Regardless, these studies clearly suggest a greater complexity in terms of maternal immunological response and regulation of endometrial cups during pregnancy.

From the previous review, it is apparent that circulating eCG concentrations vary remarkably both between and within individual mares. Factors reported to account for these fluctuations include size, parity, breed, number and genotype of fetus. However, reports are inconsistent,

thus few conclusions can be drawn. For example, higher eCG concentrations were reported in pony mares than in light horse mares (Cole, 1938; Day and Rowlands, 1940). Other investigators arrived at the opposite conclusion (Bell et al., 1967). The effects of parity, age (Day and Rowlands, 1947; Allen, 1969a) and nutrition (Clegg et al., 1962), on eCG production have not been sufficiently documented. However, mares with twin fetuses thus two sets of endometrial cups have higher serum concentrations of eCG than those with single pregnancies, a mean level of 270 IU/ml compared with 102 IU/ml respectively (Rowlands et al., 1949). Therefore, eCG production appears to be proportional to the amount of mature cup tissue present in the uterine endometrium. Fetal genotype has the most pronounced effect on eCG concentrations and its rate of disappearance from maternal blood. Manning et al. (1987) observed a sire effect on eCG concentrations which has enabled them to select sires and mares to increase endogenous eCG production. Several investigations of donkey-horse hybrid crosses showed that eCG production is depressed in mares carrying mule fetuses (mare X jack), and elevated in jennies carrying hinny fetuses (jenny X stallion). Peak serum eCG concentrations of mares carrying mule fetuses is 8 to 30 IU/ml, 10 times lower than that commonly found in mares carrying normal horse conceptuses. Furthermore, eCG concentrations drop as early as 70 to 80 d, much sooner than the 120 to 150 d disappearance expected in a normal horse pregnancy (Clegg et al., 1962; Allen, 1969c). Conversely, peak eCG levels in jenny donkeys carrying hinny fetuses range from 250 to 350 IU/ml, 5-8 times higher than the maximum levels of 30 to 50 IU/ml found in donkeys carrying normal donkey conceptuses. The cause of these

marked differences in eCG concentrations again lies in the development of endometrial cup tissue. Allen (1982) proposed that the mule conceptus develops a much narrower chorionic girdle as compared with that of the horse fetus. This results in reduced development of cup tissue, consequently lower production of eCG. In addition to reduced amount of cup tissue, the maternal leukocytic reaction against the cups is greatly increased in response to paternal antigens. By d 50 of gestation leukocytes actively invade the cup cells causing premature destruction of cup tissue and hasten the disappearance of eCG from maternal blood. In donkey jennys carrying hinny pregnancies, the chorionic girdle is much broader and more developed than in the normal donkey conceptus, giving rise to greater amounts of cup tissue consequently elevated eCG concentrations. As in the mare the leukocytic reaction is increased, however, the leukocytes are much less capable of destroying the cup tissue. Hence, the demise of the cups and subsequent disappearance of eCG is delayed.

Biological Role of eCG

Over the years, eCG has been thoroughly studied by various research groups. From their efforts understanding of its biochemical structure, endogenous levels, source and biological action in other farm species was enhanced, but the biological role of eCG in the mare remains an enigma to equine reproductive physiologists.

In other farm species, eCG has been administered as a folliculotropin to stimulate follicular development. In the mare as

well, eCG was hypothesized to have follicle stimulating activity. Its temporal relationship to the appearance of secondary follicles in pregnant mares might indicate that a causal relationship exists between the two. However, in spite of temporal relationships, recent evidence is to the contrary. Development of secondary follicles has been detected as early as 20 d of pregnancy (Bain, 1967; Allen, 1974) which precedes the formation of the fetal chorionic girdle and development of eCG-producing cells at d 37 to 40. Researchers have observed the appearance of FSH surges in early pregnancy and proposed that the appearance of waves of secondary follicles were associated with release of FSH by the pituitary between d 10 and 70 of gestation, rather than the presence of eCG (Evans and Irvine, 1975; Irvine and Evans, 1976). Squires et al. (1974) hysterectomized pregnant pony mares and observed ovarian activity in the absence of eCG. Treated mares experienced the same follicular changes as pregnant controls between d 10 and 68 of gestation. A similar study conducted by Stabenfeldt et al. (1974) produced comparable results. All pregnant mares, pseudopregnant mares and pregnant mares hysterectomized between d 10 and 68 of gestation experienced similar degrees of development of secondary follicles regardless of treatment. These studies support the general hypothesis proposed by Evans and Irvine (1975) and confirmed by Urwin and Allen (1982) that continued rhythmical release of pituitary FSH from d 10 to 70 of gestation stimulates development of secondary follicles in pregnant mares and not the presence of equine chorionic gonadotropin.

Peak ovarian activity during gestation in the mare occurs at approximately 60 d and mirrors the levels of serum eCG. This temporal

correlation between eCG levels and appearance of accessory CL has led to speculation that eCG may be the primary luteotropin involved in the luteinization of accessory follicles that appear about d 40 of gestation. Squires et al. (1975), found that intact pregnant mares, in the presence of eCG, maintained the primary CL longer (140 to 210 d) than hysterectomized mares without benefit of eCG (70 to 140 d). Additionally, in vitro studies have shown that eCG stimulated progesterone production by primary and secondary CL removed from mares at d 100 of gestation (Squires et al., 1979). These data coupled with the temporal relationship of the appearance and disappearance of eCG from the blood and the development and regression of accessory CL support the hypothesis that eCG provides the luteotropic stimulus to maintain both the primary and secondary CL in the pregnant mare.

Exogenous administration of eCG was used for induction of multiple ovulations in cows (Betteridge, 1977), ewes (Hancock and Hovell, 1961) and sows (Webel et al., 1970). Attempts to induce multiple ovulations in the mare by use of exogenous eCG were not successful (Day, 1939; Day, 1940). Dinger et al. (1972) administered intravenous and(or) intramuscular injections of eCG (1000 to 5000 IU) to progesterone-treated mares without significant effect on ovarian activity. Allen (1984) administered high dosages (20,000 to 300,000 IU, i.v.) of eCG to mares carrying transferred donkey conceptuses. Injections were at various times between d 35 and 83 of gestation. Two of eight treated mares exhibited increases in plasma progesterone as a result of secondary ovulations that occurred after three injections of eCG. The remaining six mares, however, were completely unaffected by treatment

and did not exhibit secondary ovulations. Allen argued that exogenous eCG can induce secondary ovulations but it must be given at times when sufficiently mature secondary follicles are able to respond to the proposed LH-like activity of eCG. Additionally, Allen suggested that eCG alone may not be luteotropic, but combined with other luteotropins, possibly of fetal or pituitary origin, may act synergistically to stimulate secondary ovulations, follicular luteinization and maintenance of accessory CL.

The eCG beta subunit shares 85% homology with LH-like hormones and displays luteotropic activity in vitro. In vivo studies conducted by Squires et al. (1974) demonstrated that pregnant pony mares hysterectomized between d 10 and 68 of gestations, in the absence of eCG, failed to ovulate or luteinize secondary follicles by d 70. In the same study, 12 of 17 intact pregnant pony mares exhibited secondary ovulations. Accumulated evidence on the biological role of eCG implies that the luteotropic action of endogenous eCG is: eCG prolongs the life span and stimulates secretory activity of primary CL, and eCG alone or synergistically with pituitary gonadotropins induces ovulation and(or) luteinization of mature secondary follicles during pregnancy.

To date, the physiochemical and structural characteristics of eCG have been identified, yet no concrete information has been presented characterizing its specific receptors or biological mechanism of action. Limited studies have shown that eCG is capable of binding to both FSH and LH receptors in many different tissues and species. Specific radioreceptor assays developed by Stewart et al. (1976), were used to measure FSH-like and LH-like activities of eCG by use of tissue

receptors from rat testes. In mares, the ratio of the two activities did not vary significantly among individuals or stages of gestation. Equine chorionic gonadotropin demonstrated both FSH and LH activity in rat tissue, however, the FSH-like activity of eCG in serum exceeded its LH-like activity by a factor of approximately 1.5. Based on radioreceptor assays, Stewart and Allen, (1979) illustrated that the relative numbers of FSH receptors and LH receptors were similar in both equine and non-equine gonadal tissues. Equine chorionic gonadotropin, human chorionic gonadotropin (hCG), LH and FSH bound non-equine receptors with the same efficiency on a per molar basis. In contrast, eCG was much less efficient than hCG and pituitary gonadotropins at binding to equine gonadotropin receptors. Equine chorionic gonadotropin binding, measured as a percentage of LH binding, was 89 to 103% in non-equine tissue and only 2 to 4% in equine tissue. Competitive binding studies revealed that eCG binds to equine LH receptors with about one tenth the affinity of LH and fails to bind to FSH receptors. In a similar study using radioreceptor assays, Licht et al. (1979) reported that binding activity of eCG was only 1 to 5% of equine LH in competing for FSH receptors, and only 3 to 35% of equine LH in competing for LH binding sites in equine tissue. These studies were interpreted to indicate that eCG displays LH-like activity in the mare by binding to LH receptors. Additionally, Stewart and Allen (1979) noted that although eCG binds to equine tissues with a lower affinity than other gonadotropins it may be no less effective in producing luteotropic results because of the tremendous amounts of circulating hormone in the mare (maximum blood content 3 million IU). Stewart and Allen (1979)

proposed that the mare has developed a refractory tolerance to eCG that might explain the decreased binding affinity of eCG for LH receptors. Conversely, intrinsic differences in receptors between species might explain the dual FSH/LH activity of eCG in various species. Regardless of conjecture, information from radioreceptor studies additionally confirms the biological significance of eCG as a hormone with a luteotropic function to induce ovulation and maintain the primary and secondary CL in pregnant mares.

Use of eCG in Pregnancy Diagnosis

Since Cole and Hart (1930) first indicated the diagnostic value of using equine chorionic gonadotropin for detection of pregnancy, several commercial and laboratory pregnancy tests were developed for this purpose. The earliest of these, a bioassay called the "mouse test," involved injecting pregnant mare serum into weanling mice, which in the presence of eCG stimulated uterine hypertrophy and follicular growth with 90% reliability (Cole and Hart, 1930). Wide and Wide (1963) described a hemagglutination-inhibition test that produced results within a few hours with 97% reliability as early as d 41 to 45 of gestation. Modern laboratory and field tests were based on enzyme-linked immunosorbent systems (ELISA). Using ELISA tests various manufacturers report 100% reliability detecting eCG between d 40 and 80 of gestation. Although these tests are quick and easy to use their results should not be considered conclusive because research has shown that mares that abort after the establishment of endometrial cups

continue to produce eCG as if they were still pregnant. Persistent endometrial cups continue to secrete gonadotropin until their natural degeneration about d 130 to 150 after conception (Catchpole and Lyons, 1934; Cole and Hart, 1942; Allen, 1969c). Consequently, in such cases eCG pregnancy tests frequently give false-positive results in which the presence of eCG is indicated but the mare may not be pregnant. These results can only be verified by rectal palpation and(or) ultrasound examination to determine the true status of the pregnancy. Mitchell (1971) illustrated this point in a study which reported that 23 of 62 eCG pregnancy tests conducted after the time of fetal death indicated false-positive test results. In Mitchell's study false-positive test results were obtained to 65 d after fetal death. Because onset and decline of eCG production may differ between mares, pregnancy tests administered before 40 d or after 90 d of gestation may provide false negative results as endometrial cups may not be fully established. For accurate and consistent results, manufacturers recommend testing for eCG between d 40 and 80 of gestation when eCG concentrations are at a peak.

Equine Chorionic Gonadotropin Dominated Pseudopregnancy

As reviewed by Ginther (1979), various authors reported 5 to 30% incidence of early pregnancy loss of established pregnancies. At the time of endometrial cup formation, d 30 to 35, pregnancy losses of 29.3% (Villahoz et al., 1985) and 4.2% (Ginther et al., 1985) were reported. Only 4.3% of the losses occur between d 35 to 40 (Ginther et al., 1985). Recent studies based on scanning ultrasound provided more reliable

information on the incidence and time of embryonic loss. However, these figures must be interpreted with caution because of divergent examination programs, different reference points and(or) the use of subfertile, embryo transfer and normal mares among studies. Factors responsible for early embryonic death in mares include: nutrition, photoperiod, plant estrogens, prebreeding seminal treatments, genital infections, chromosomal abnormalities, hormonal deficiencies, stress, failure of physiological recognition of pregnancy and(or) placentation, and lactational stress and foal heat breeding (Ginther, 1979).

In the event of abortion after the establishment of endometrial cups, the function of the cups is retained and they continue to secrete eCG (Clegg et al., 1954). Upon surgical removal of the conceptus on or after d 38 of gestation (Allen, 1970) or in the event of fetal death after d 40 (Mitchell, 1971), eCG secretion continues normally from d 35 to 150, indicating that the functioning life span of the cups is independent of the conceptus. Mares that undergo loss of pregnancy while endometrial cups are present often fail to resume normal cyclic activity and frequently exhibit anovulatory estrus (Allen, 1978; Squires et al., 1980), prolonged luteal function (Thompson et al., 1982), ovulation without estrus or anestrus with small, nonfunctional ovaries (Squires et al., 1980). Irregular cyclic behavior following abortion frequently is referred to as "eCG dominated pseudopregnancy," a syndrome of anovulatory estrus that occurs when the ovaries are under the influence of eCG. The absence of regular estrous cyclicity after abortion impairs the opportunity for rebreeding during the same breeding season.

It is possible that development of endometrial cups and secretion of eCG influence ovarian activity and reconception in aborted mares. Several studies have been conducted to determine these effects in mares aborted before or after formation of endometrial cups. Various techniques to remove the conceptus have been employed in these studies with varied degrees of success. Crushing the fetus, aspiration of placental fluid, administration of hCG, surgical removal and intrauterine saline infusions are all techniques used to terminate pregnancy when administered before d 36 of gestation (Allen, 1978). However, few of these methods induce complete luteolysis, therefore, abortions often are accompanied by maintenance of a functional CL and the mare fails to return to estrus for 33 to 54 d (Kooistra and Ginther, 1976; Allen, 1978). A single injection of prostaglandin $F_2\alpha$ on d 32 in ponies (Kooistra and Ginther, 1978) and d 35 in horses (Squires et al., 1980) resulted in complete luteolysis, indicated by low progesterone levels, abortions in 2 to 4 d and return to ovulatory estrus in 15.2 ± 7.2 d. In the presence of eCG, removal of the conceptus may or may not result in maintained luteal function in individual mares (Allen, 1975b). Abortion is more difficult to induce with $PGF_2\alpha$ and return to ovulatory estrus is greatly delayed after endometrial cups are fully functional. The presence of secondary CL at this time might be implicated as the cause of the failure to resume normal estrous cycles. Squires et al. (1980) showed that a single dose of a $PGF_2\alpha$ analogue administered at d 70 of gestation failed to induce abortion in 8 of 8 mares but repeated injections administered at 12 or 24 h intervals successfully terminated pregnancy in 16 of 16 mares at d 70. In both studies, repeated

injection of $PGF_2\alpha$ from d 42 to first estrus resulted in abortion and return to ovulatory estrus. Repeated dosages resulted in complete luteolysis of both primary and secondary CL as indicated by reduced progesterone concentrations but eCG concentrations were not altered. In addition, Squires et al. (1980) reported that 12 of 16 mares exhibited a period of anovulatory estrus and 4 of 16 ovulated without estrus. Normal estrus and ovulation typically were delayed 40 to 50 d. Results of these studies showed that although repeated administration of $PGF_2\alpha$ achieved luteolysis, luteolysis in itself did not correct the irregular cyclic behavior characteristic of eCG dominated pseudopregnancy. Mares in this and other similar studies continue to ovulate with or without estrus ultimately reducing their ability to reconceive in the same breeding season. The net effect is that normal cyclicity is not restored until endometrial cups disappear around 4 months after the original conception date. Allen (1975b) speculated that the LH-like activity of eCG induces continued ovulation and luteinization of secondary follicles which in turn produce the pseudopregnant state. Thompson et al. (1982) argued that because eCG levels are extremely high and not timed properly with the mares cycle or follicular growth, the eCG may prevent synchronization of normally cyclic endocrine events thus contribute to the pseudopregnant state. Consequently, the literature is clouded on the effect of eCG on the ovaries of pregnant and nonpregnant mares. More study is needed on the biological action of eCG and perhaps more importantly what factors are responsible for signaling the cups to cease their secretion of eCG and to degenerate.

Monoclonal Antibody to eCG

Passive immunization was used to suppress the folliculotropic activity of eCG in cattle. Simultaneous administration of eCG and anti-eCG serum were shown to successfully block eCG action and control superovulation (Dhondt et al., 1978; Kim et al., 1987). Because of its increased availability and specificity, hybridoma-derived monoclonal antibodies now are frequently employed in endocrine studies for passive immunization and more often are used to replace conventional antiserum in immunodiagnostic tests.

The monoclonal antibody (518B7) described by Matteri et al. (1987) was generated against the beta subunit of bovine LH. It has demonstrated high specificity for a variety of mammalian LH preparations including eCG and eLH. Although cross reaction with eCG (38.7%) was less potent than with eLH (100%) enough cross reactivity was reported to exist to use the monoclonal antibody to measure eCG concentrations during pregnancy.

Spearow and Trost (1987) developed and validated a sensitive ELISA for cattle, sheep, rat and mouse LH by use of the monoclonal antibody (518B7) antibovine βLH. With the same antibody, a dipstick ELISA test to detect eCG and eLH in mares was developed by Kasper et al. (1987). The 30 min dipstick test proved capable of detecting eCG with 98 to 100% reliability from d 40 to 99 of gestation.

INTRODUCTION TO EXPERIMENTS

Endometrial cups are temporary glands which form in the uterus of the pregnant mare and secrete eCG from d 35 to 120 of gestation (Cole and Goss, 1943; Clegg et al., 1954; Allen and Moor, 1972). In the event of termination of pregnancy after establishment of endometrial cups, the function of the cups is retained and they continue to secrete eCG to d 120 (Allen, 1975b). Consequently, mares in which pregnancy is terminated while the endometrial cups are present often fail to resume normal cyclic activity until the endometrial cups have degenerated and eCG is no longer produced (Douglas et al., 1974; Allen, 1978; Rathwell et al., 1987). Absence of normal estrous cycles following abortion reduces the opportunity for rebreeding during the same breeding season.

Experiments were conducted to determine if passive immunization against eCG would: reduce plasma eCG concentrations, cause notable changes in ovarian activity with respect to accessory ovulations, follicular luteinizations and CL maintenance, and facilitate resumption of normal estrous cycles after early abortion when eCG normally is present.

MATERIALS AND METHODS

Purified mouse monoclonal antibody (MAb, clone 0220-518B7 antibovine βLH)[1] was used for passive immunization and eCG assays. The antibody has demonstrated specificity for the beta subunit of eLH and eCG and has been used in both radioimmunoassay (Matteri et al., 1987) and ELISA to detect LH in other species (Spearow and Trost, 1987) and eCG in horses (Kasper et al., 1987).

Experiment I

Clinical and hormonal data were collected from nine pony mares from April 1 to September 30, 1987. Animals were housed at the Oregon State University Horse Center, Corvallis, Oregon. All feeding and maintenance care was conducted by Horse Center staff. All mares were cycling normally at the onset of the experiment. Their history of parity was unknown. Individual mares were teased daily with a stallion as described by Ginther (1979). Mares with follicles > 25 mm were bred by natural service or artificial insemination using a stallion of proven fertility. Day 1 postovulation was designated d 1 of gestation. All mares were confirmed to be pregnant by palpation and ultrasonography using a 5.0 Mhz transducer[2] on d 30 and randomly assigned to one of three groups. Treatment of mares was as follows: Group C, control mares (n=3); Group

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[1]Provided by Monoclonal Antibodies, Inc., Mountain View, CA 94043

[2]Model #210 DX, Corometrics Medical Systems, North Branford, CT 24358

PGF, (n=2) prostaglandin $F_2\alpha$ ($PGF_2\alpha$, 5 mg, i.m.)[3], on d 45 and every 12 h until abortion; Group PGF/MAb, (n=4) $PGF_2\alpha$ as in group PGF plus monoclonal antibody (35 mg, i.v.) on d 45 and 49. All mares were teased, palpated and examined via ultrasound daily for detection of estrus, follicular development and accessory ovulation from d 45 to 65, at 2 d intervals from d 65 to 85 and once weekly thereafter to d 100. Termination of pregnancy was defined as loss of fetal heartbeat and(or) disorganization of fetal tissues. Aborted mares that displayed estrus and possessed a follicle > 20 mm were inseminated with semen collected from a fertile stallion and examined by palpation and ultrasound for pregnancy 15 d postovulation.

Jugular vein blood samples were sequentially collected to assay circulating plasma eCG and progesterone concentrations. Pretreatment blood samples were collected at four day intervals from d 35 to 45. Monoclonal antibody and(or) prostaglandin were given at time 0 on d 45. Post-treatment samples were collected at 2, 4, 8 and 12 h. Thereafter, samples were collected daily from d 45 to 55, alternate days from d 55 to 65, every four days from d 65 to 85 and once weekly through d 100. Blood was collected into 10 ml evacuated heparinized tubes and centrifuged for 15 min at 1500 x g within 30 min of collection. Plasma was stored in plastic vials at -20°C until assayed.

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[3]Lutalyse, The Upjohn Co., Kalamazoo, MI 49001

Experiment II

Six pregnant pony mares were randomly assigned to either: untreated pregnant controls (C, n=3), or pregnant mares (MAb, n=3) treated once daily with monoclonal antibody on d 35 (25 mg, i.v.), 36 (35 mg), 37 (45 mg), and 38 (55 mg). All mares were housed, bred, and confirmed pregnant as described previously. Following treatment, mares were teased, palpated and examined via ultrasound. Data were collected daily on ovarian volume, accessory follicular development, accessory ovulation and luteinization and fetal growth from d 35 to 65, alternate days from d 65 to 85 and every four days thereafter through d 100 of gestation.

Pretreatment blood samples were collected at 2 d intervals from d 30 to 35. Monoclonal antibody was administered at time 0 on d 35. Blood was sampled at 2, 4, 8, and 12 h on d 35, daily from d 35 to 45, at 2 d intervals from d 45 to 65, at 4 d intervals from d 65 to 85 and once weekly thereafter to d 100. Plasma samples were processed and stored as described previously.

Hormone Assays

Progesterone

Progesterone concentrations in plasma were measured by the radioimmunoassay method validated and described by Koligian and Stormshak (1977). Duplicate samples were extracted with petroleum ether once. Recovery of progesterone was determined by extracting an

additional sample to which 20,000 dpm [^{3}H-] 1,2,6,7-progesterone had been added to correct for procedural losses. The working range of the standard curve was between 5 and 1000 pg/tube. Assay sample volumes were no less than 25 μl, therefore, an overall sensitivity of at least .1 ng/ml or better was achieved. The intra- and interassay coefficients of variation were 8.5% and 20.3%, respectively.

Equine Chorionic Gonadotropin

Plasma eCG concentrations were determined by an enzyme-linked immunosorbent assay. A solution containing mouse monoclonal antibody (518B7 antibovine βLH, 10 μg/ml) in phosphate buffered saline (PBS, pH = 7.0) was generated to coat the antibody onto Linbro/Titertek plates[4]. One hundred microliters were added to each well. Plates were covered and incubated at room temperature overnight and then washed twice with wash solution (0.04 gm/100 ml Tween 20, 0.01 gm/100 ml Thimersol dissolved in 100 ml PBS, pH = 7.0). Varying volumes of sample or standard sera were added to wells in triplicate. Sera samples were diluted with 1% BSA-PBS and 50 μM Tris-HCl (pH = 7.0), respectively, to achieve a total volume of 50 μl per well. Buffer controls consisted of 50 μl of 50 mM Tris-HCl (pH = 7.0). Plates were incubated overnight, washed twice and 50 μl of antiequine αeCG alkaline phosphatase conjugate[5] were added (diluted 1:250 in 50 mM Tris-HCl buffer, pH = 7.0 containing 0.5 mg/ml of

[4]Flow Laboratories, Inc., N. Hollywood, CA 91605.

[5]Monoclonal Antibody Inc., Mountain View, CA 94043

nonspecific IgG). After 2 h of incubation at room temperature, plates were washed twice and 50 μl of p-nitrophenyl phosphate (1 mg/ml in 50 mM Tris-HCl, pH = 10 with 1 mM $MgCl_2$) were added to all wells containing samples. One hundred microliters of water were added to blank wells. The reaction was allowed to proceed for 30 min at room temperature, after which 50 μl of stop solution (50 mM EDTA, pH = 12) were added to all wells. Optic density was determined at 405 nm by use of a Bio-Tek EL-307 ELISA plate reader[6].

The working range of the standard curve was between .1 and 6.0 ng. Recoveries of added amounts of standard sera to given volumes of plasma were 70%. Assay sensitivity was .1 ng/tube. Given that 50 μl was the maximum volume used, overall sensitivity was at least 2 ng/ml or .03 IU/ml. Equine chorionic gonadotropin is reported in international units (where .015 IU equals 1 ng protein). The intra- and interassay coefficients of variation were 25.0% and 42.0%, respectively. Because of these variabilities, this assay was considered semiquantitative. However, all samples from an individual mare were analyzed in a single assay. These data (eCG and progesterone) were analyzed by split-plot analysis of variance[7].

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[6]Bio-Tek Instruments, Inc., Highland Park, Box 998, Winooski, VT 05404-0998

[7]SAS Institute Inc., Cary, NC 27512

RESULTS AND DISCUSSION

Experiment I

A normal increase in plasma concentrations of eCG occurred in control mares beginning on d 39 (Figure 1). No significant differences were found between plasma eCG or progesterone concentrations in mares treated with $PGF_2\alpha$ alone (PGF) or with antibody (PGF/MAb). Consequently, data from these two treatment groups were combined and compared with that of controls. A transient plateau or slight decrease in plasma eCG concentrations occurred in treated mares from 2 to 24 h on d 45 ($P < .12$). On d 46, 47, 48 and 51 eCG levels were significantly lower in treated mares ($P < .05$). Thereafter, plasma eCG concentrations increased in a pattern similar to that of control mares. Although the initial dose of MAb was sufficient to temporarily reduce circulating eCG between d 45 and 48, by d 49 endogenous eCG levels apparently were too high to be effectively reduced by the initial dosage of antibody. One pregnant control mare exhibited extremely high plasma eCG concentrations (500 to 700 IU/ml) from d 51 to 77.

Mares in both treated groups (PGF and PGF/MAb) showed a similar decrease in plasma progesterone ($P < .05$) from 2 h post treatment on d 45 to d 61 (Figure 2). Presumably, repeated $PGF_2\alpha$ treatment induced rapid luteolysis with a consequent reduction in progesterone secretion. Unusually high progesterone concentrations were observed in one control mare and one PGF mare (Figure 3) from d 55 to 96. Upon palpation and ultrasound examination, ovaries of both mares were enlarged,

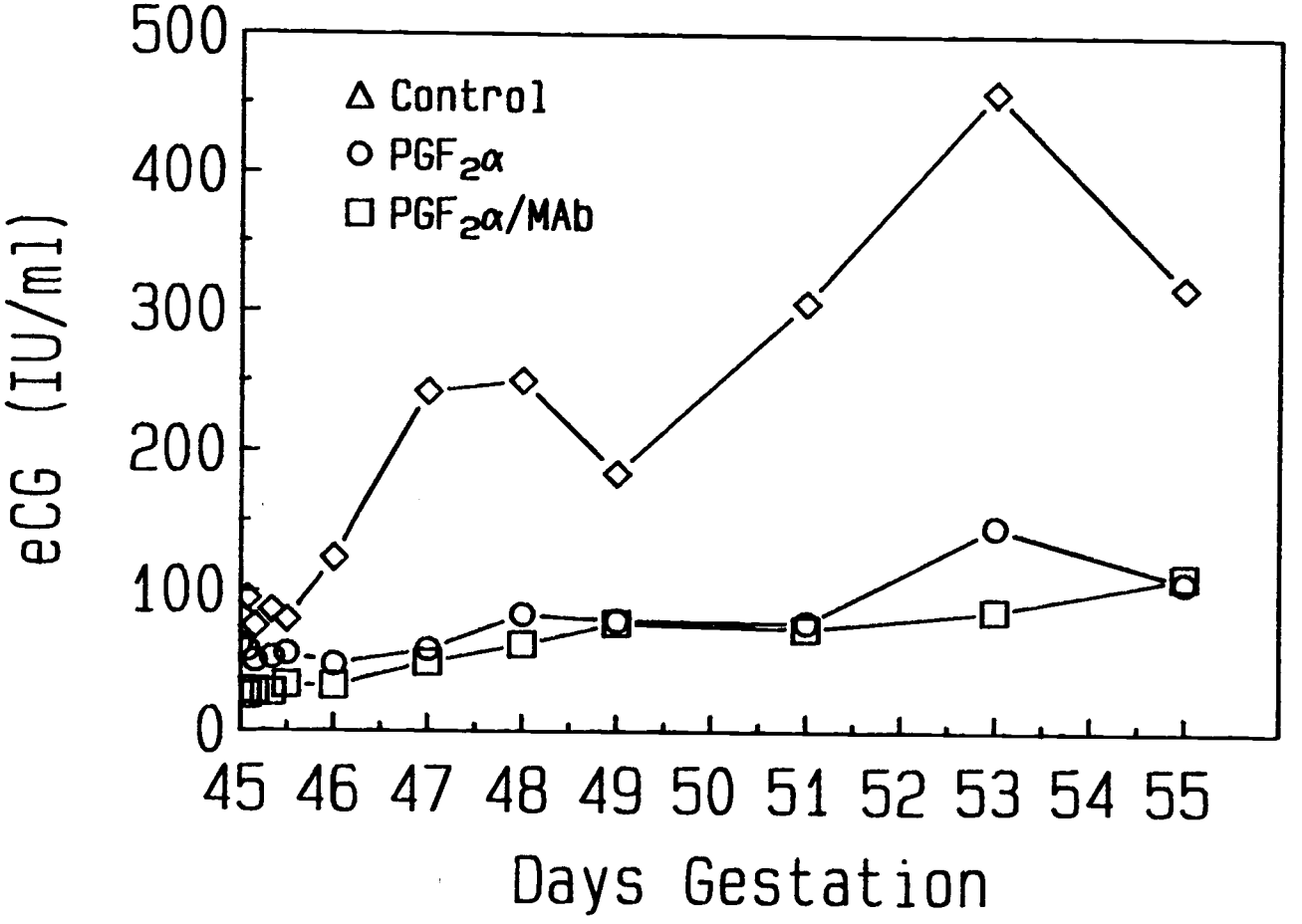

Figure 1. Plasma equine chorionic gonadotropin (eCG) in control mares (C), mares treated with prostaglandin $F_2\alpha$ ($PGF_2\alpha$) on d 45 and every 12 h until abortion and mares treated with $PGF_2\alpha$ (every 12 h) plus monoclonal antibody ($PGF_2\alpha$/MAb) on d 45 and 49. Plasma eCG concentrations in treated mares were less than controls on d 46, 47, 48 and 51 ($P < .05$, common standard error = 45.8).

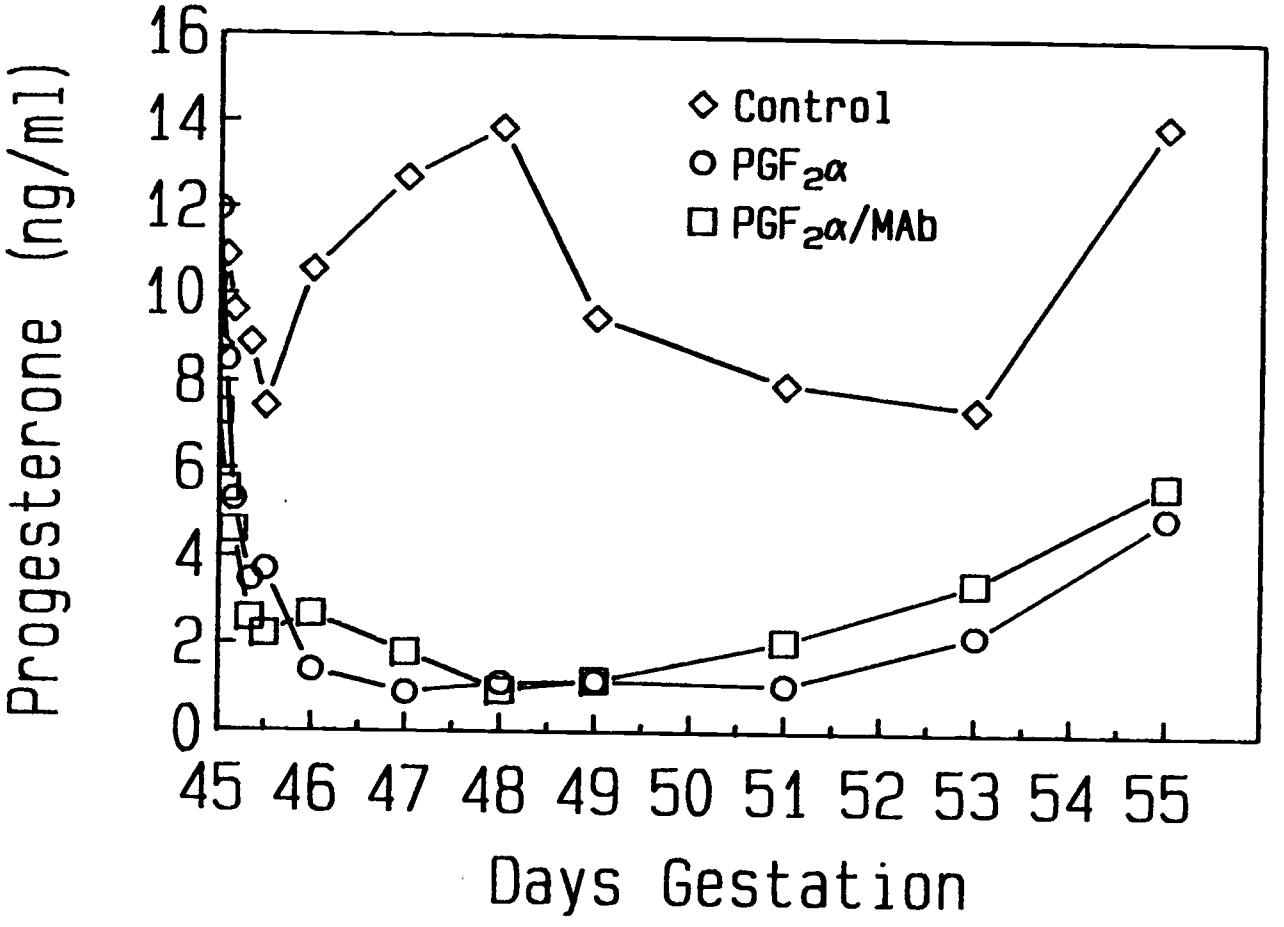

Figure 2. Plasma progesterone in control mares (C), mares treated with prostaglandin $F_2\alpha$ ($PGF_2\alpha$) on d 45 and every 12 h until abortion and mares treated with $PGF_2\alpha$ (every 12 h) plus monoclonal antibody ($PGF_2\alpha$/MAb) on d 45 and 49. Plasma progesterone concentrations in treated mares were less than controls from 2 h post treatment on d 45 to d 61 ($P < .05$, common standard error = 1.7).

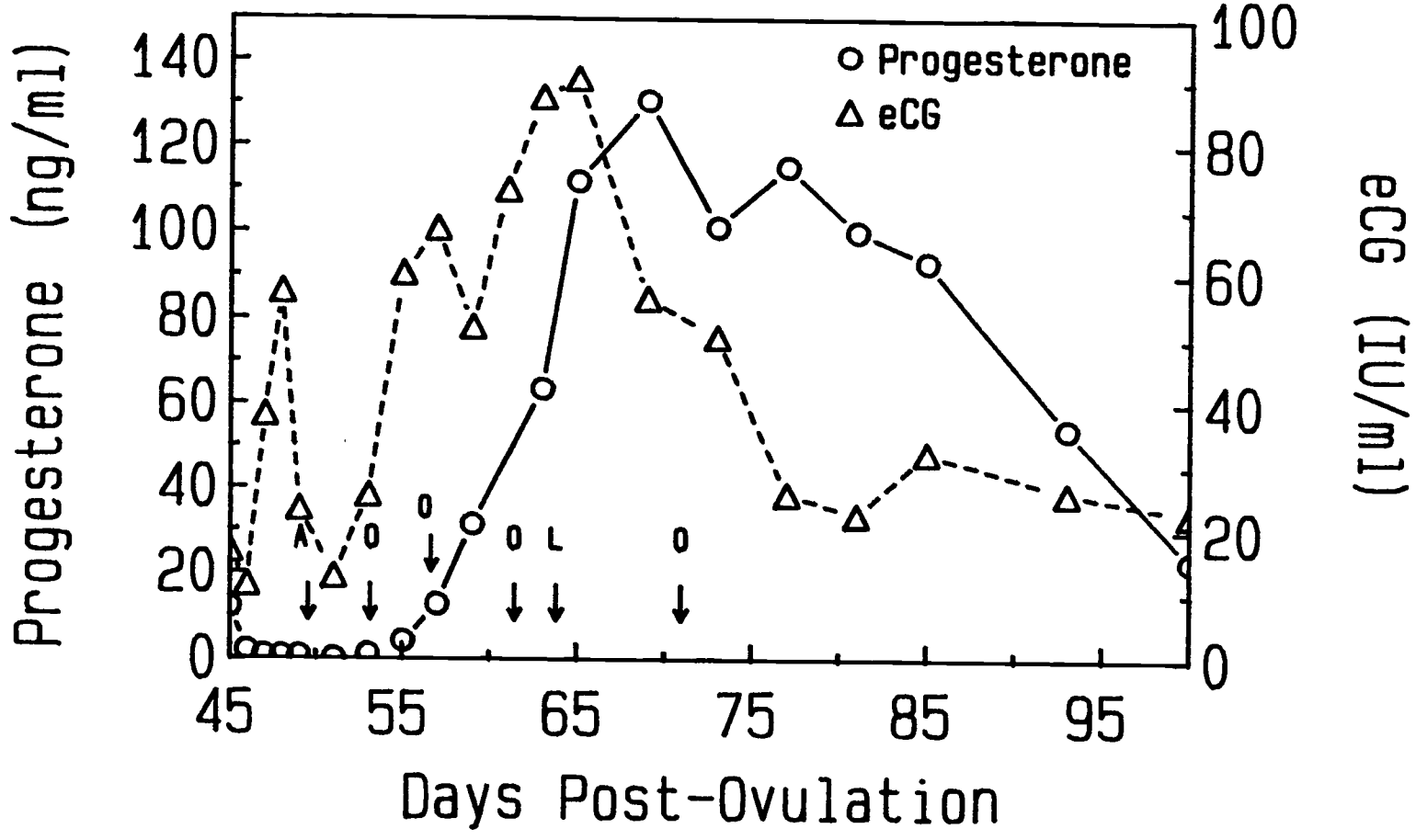

Figure 3. Changes in plasma eCG and progesterone concentrations in a mare given prostaglandin $F_2\alpha$ on d 45 and every 12 h until abortion. Ovulatory estrus occurred between d 47 and 52. Arrows indicate abortion (A), ovulatory CL (O) and luteinized follicles (L). Note the difference in scale.

reaching 10 to 12 cm in both length and width. Increased ovarian activity was reflected in plasma progesterone concentrations which exceeded 100 ng/ml from d 65 to d 85. Plasma eCG concentrations reached a maximum value of 70 IU/ml in the control mare and 135 IU/ml in the mare treated with $PGF_2\alpha$. Although it is speculated that eCG is luteotropic in the mare, these data indicate that ovarian activity is not dependent upon high concentrations of circulating hormone. In this regard, Allen (1984) proposed that eCG alone is not luteotropic but in combination with another hormone, possibly of fetal or pituitary origin, eCG may function synergistically to induce secondary ovulation, follicular luteinization and maintenance of accessory CL.

Mean number of days from treatment to abortion (5.8 d), abortion to first estrus (9.5 d), duration of estrus (4.8 d) and presence of ovulatory estrus were highly variable among mares and did not differ significantly between PGF and PGF/MAb groups. Three of the six treated mares exhibited ovulatory estrus subsequent to abortion. A fourth mare ovulated 3 d post-estrus. The two remaining mares failed to exhibit estrus and(or) exhibited ovulation without estrus. None of the treated mares became pregnant following breeding and plasma concentrations of eCG remained elevated until after d 100. Results of the present study are consistent with those of other studies (Squires et al., 1974; Penzhorn et al., 1986; Rathwell et al., 1987). However, in addition, two of the treated mares exhibited estrus before abortion. Mares undergoing loss of pregnancy while endometrial cups are present often exhibit irregular cyclic behavior characterized by periods of anovulatory estrus (Allen, 1978; Squires et al., 1980), prolonged

diestrus (Thompson et al., 1982) ovulation without estrus or anestrus accompanied by small nonfunctional ovaries (Squires et al., 1980). Squires et al. (1980) suggested that in the presence of eCG, repeated administration of $PGF_2\alpha$ to induce abortion achieves complete luteolysis but luteolysis in itself does not appear to correct the irregular cyclic events observed in the post-abortion mare. One mare treated with $PGF_2\alpha$ in the present study displayed similar irregular cyclic characteristics (Figure 3). Estrus occurred from d 47 to 52. Abortion occurred on d 49. Estrus was accompanied by ovulation on d 51 but conception did not occur. Accessory ovulations and luteinizations occurred without estrus on d 56, 68, 69 and 71. After the 100 d test period the mare exhibited ovulatory estrus from d 107 to 114 to which she conceived. By d 133 (d 19 of gestation) plasma eCG from the previous aborted pregnancy was still detectable by use of an eCG dipstick ELISA assay system. Another mare treated with $PGF_2\alpha$/MAb exhibited a similar pattern (Figure 4). Ovulations and luteinizations occurred both with and without estrus. Ovulatory estrus was observed on d 75 and 95; conception occurred only at the second estrus.

In the presence of eCG, treated mares had fewer ($P < .13$) accessory CL (5.2) than control mares (8.0) from d 35 to 100. Reduced eCG concentrations observed in treated mares between d 45 and 51 may account for fewer accessory CL which begin to appear about d 40 of gestation. However this causal relationship is not conclusive, because all treated mares also received $PGF_2\alpha$ (every 12 h) from d 45 to abortion (5.8 d $\pm$ 1.0 days SE). Within the present study, it was not determined if reduced number of ovulations and luteinizations in treated mares were

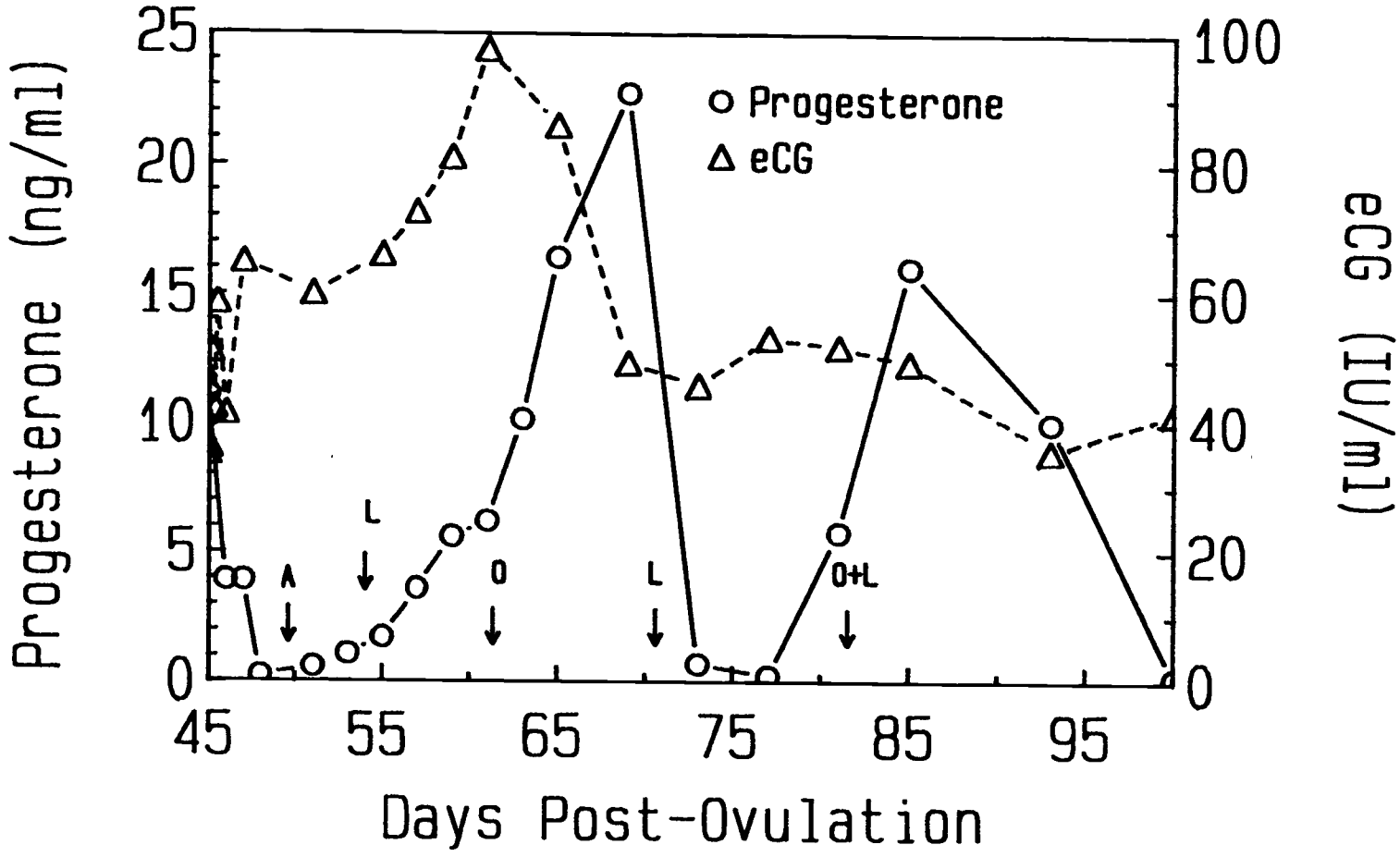

Figure 4. Changes in plasma eCG and progesterone concentrations in a mare given prostaglandin $F_2\alpha$ on d 45 every 12 h until abortion plus monoclonal antibody on d 45 and 49. Estrous behavior was observed on d 49 and 50; arrows indicate abortion (A), ovulatory CL (O), and luteinized follicles (L). Ovulatory estrus occurred at 75 and 95 d; conception occurred at the second estrus.

the effect of MAb alone or in combination with $PGF_2\alpha$. Others have reported that repeated injections of $PGF_2\alpha$ starting on d 42 (Rathwell et al., 1987) and d 70 (Squires et al., 1980) did not affect secretion of eCG. If endogenous eCG is luteotropic in the mare then any reduction in its concentration related to treatment might account for fewer accessory ovulations and luteinizations. These data are not in agreement with those of Allen (1984) who reported, that although accessory ovulations occur in the presence of eCG, the number of accessory CL and subsequent progesterone secretion are not correlated with absolute levels of eCG. The two mares previously discussed in this study have illustrated this point. Numerous secondary ovulations and luteinizations observed between d 55 to 96 resulted in plasma progesterone concentrations > 100 ng/ml whereas eCG concentrations did not exceed 100 IU/ml. It also might be postulated that the presence of circulating antibody complicated binding of eCG or LH to ovarian receptors thus reducing the incidence of accessory ovulation at the onset of treatment (d 45).

Experiment II

Daily treatment with increasing amounts of MAb on d 35 to 38 of pregnancy did not affect plasma eCG concentration in treated vs control mares during this interval (Figure 5). However, by d 39, mean plasma eCG concentrations were significantly lower ($P < .05$) in treated mares. Presumably, at this time sufficient quantities of circulating antibody had accumulated to prevent the increase in plasma eCG observed in untreated pregnant controls. Thus, increasing amounts of antibody given daily appeared to block endogenous concentrations of eCG during the early stages of formation of endometrial cups and secretion of eCG. After d 40, concentrations of eCG in treated mares increased in a pattern similar to that of controls suggesting that eCG concentrations exceeded circulating antibody concentrations. In one pregnant control mare, plasma eCG was detected on d 34. This early appearance of eCG preceded that of all other controls in both studies.

Plasma progesterone in antibody-treated mares decreased ($P < .05$) on d 36 only (4.18 vs 7.83 ng/ml in controls; Figure 6). Thereafter, progesterone concentrations increased in mares of both groups and did not differ between control and treated animals.

Control of the formation, maintenance and regression of the secondary CL generally is attributed to the presence of eCG. In this regard, in controls 78% of CL were from ovulatory follicles and 22% were from unruptured follicles that luteinized. This ratio was reversed in treated mares such that 30% were from ovulatory follicles and 70% were

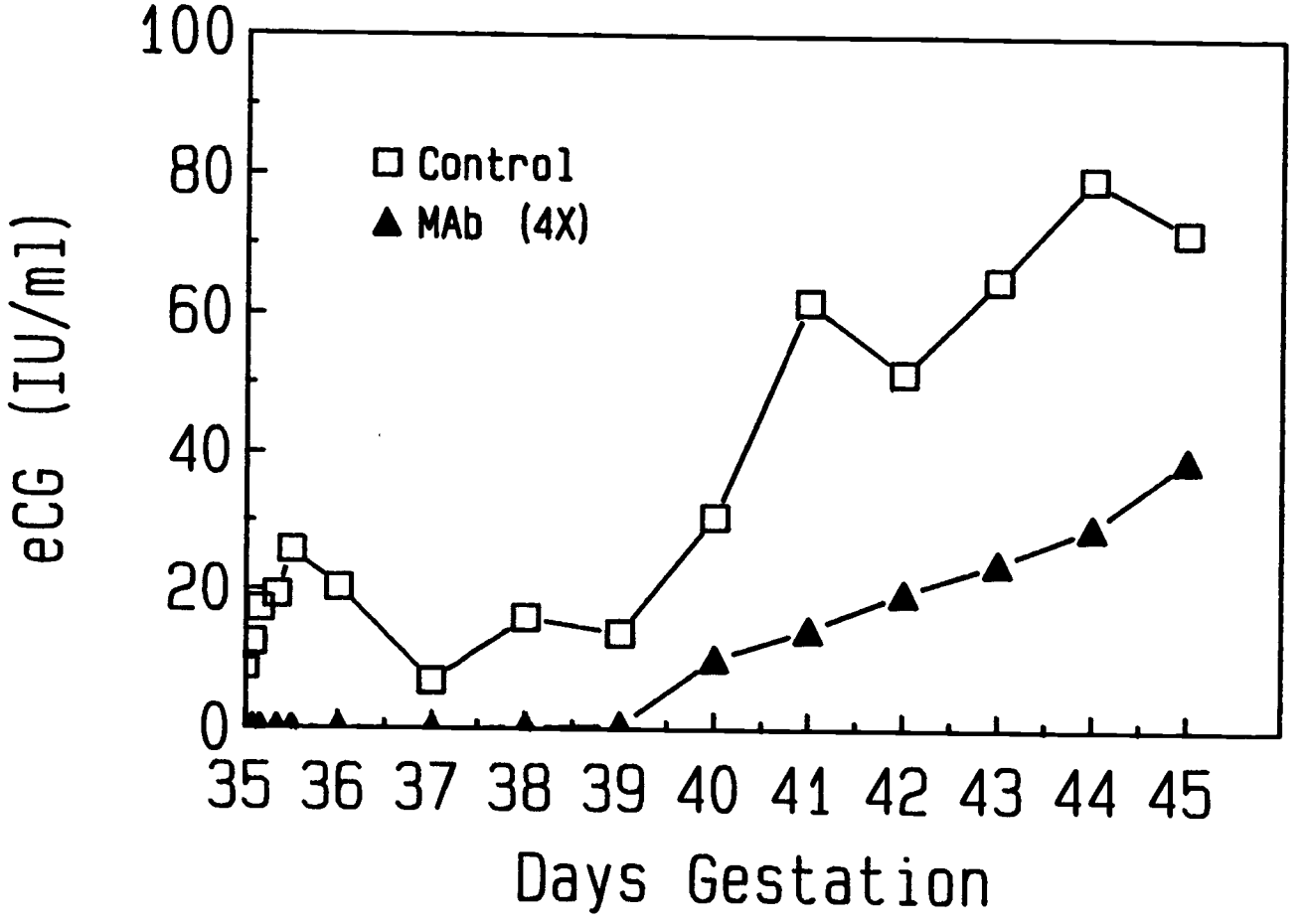

Figure 5. Plasma equine chorionic gonadotropin (eCG) concentrations in control mares (C) and mares treated with monoclonal antibody (MAb) on d 35 (25 mg), 36 (35 mg), 37 (45 mg) and 38 (55 mg). Compared to control mares, plasma eCG concentrations were lower in treated mares on d 39 only ($P < .05$, common standard error = 6.9).

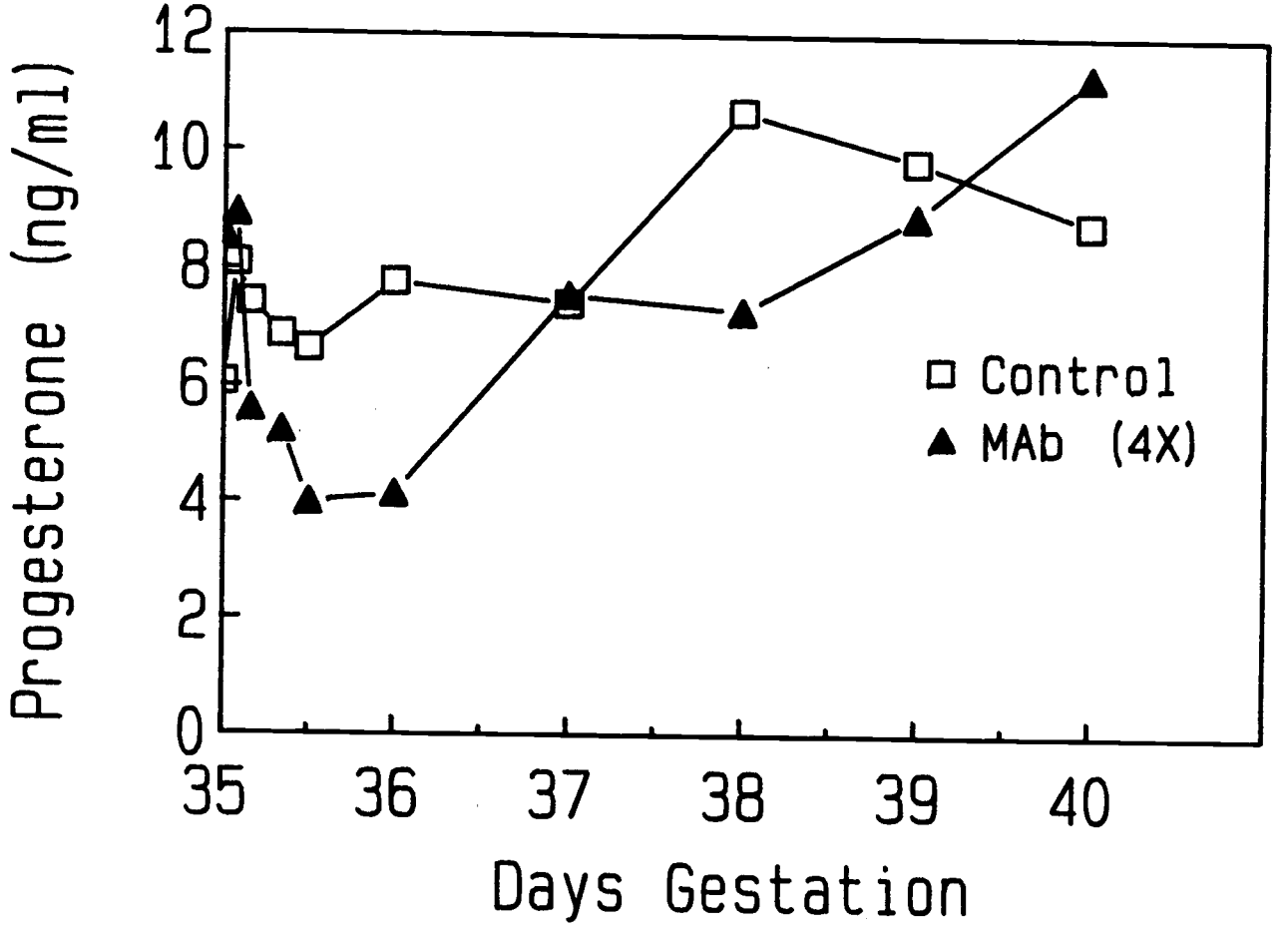

Figure 6. Plasma progesterone concentrations in control mares (C) and mares treated with monoclonal antibody (MAb) on d 35 (25 mg), 36 (35 mg), 37 (45 mg) and 38 (55 mg). Plasma progesterone was lower ($P < .05$, common standard error = 1.33) in treated mares on d 36 only.

from unruptured luteinized follicles. The overall number of CL was not different between groups and this was reflected in similar plasma progesterone concentrations. By ultrasonography, luteinized follicles were observed developing during a period of 2 to 3 d. Normal appearing, fluid-filled follicles gradually developed fine echogenic lines and eventually acquired a grey, mottled appearance. These quickly developed into echogenic structures recognized as mature CL. Greater plasma progesterone concentrations were closely correlated with the appearance of these structures. Time of the first accessory CL formation was delayed in treated mares (71.1 $\pm$ 4.4 d) compared to controls (57.7 $\pm$ 4.5 d). These findings suggest that lower concentrations of eCG during the period of cup development did not prevent secondary luteinizations but possibly were a contributing factor in their delayed ovulation or the increased incidence of luteinized follicles. The long term significance of this is not known because eCG levels of treated mares after d 40 were similar to those of controls.

CONCLUSIONS

Passive immunization of aborted mares on d 45 and 49 at the given dosages did not reduce plasma eCG concentrations significantly. Temporary decreases in plasma progesterone and a tendency for fewer accessory CL to develop following abortion may be attributed to the single or combined effect of $PGF_2\alpha$ and MAb. Treatment did not facilitate resumption of normal estrous cycles as indicated by failure of half of the treated mares to return to ovulatory estrus and failure of all treated mares to conceive following abortion.

Repeated administration of MAb to pregnant mares during early development of endometrial cups appeared to reduce circulating eCG until endogenous eCG concentrations exceeded the levels of exogenous antibody. Treatment had a transient effect on progesterone secretion (24 h). Accessory corpora lutea formation was delayed and a tendency toward increased anovulatory luteinization was observed in pregnant treated mares. With respect to the biological action of eCG, the continued use of MAb for hormone receptor studies should be considered.

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