NAB 365 (CLENBUTEROL, PLANIPART™) FOR THE POSTPONEMENT OF PARTURITION AND ALLEVIATION OF DYSTOCIA IN CATTLE

By

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PREFACE

This study was done for Boehringer Ingelheim Inc. Boehringer Ingelheim Inc. is submitting this drug to the United States Department of Food and Drug Administration for use in the United States. The primary objective is to document efficacy reported in European studies.

The author wishes to thank Boehringer Ingelheim Inc. for granting this trial to my major adviser Dr. Lawrence Rice and Oklahoma State University. Appreciation is also given to Dr. Rice for his help, leadership, and guidance during my master's work.

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THE RESEARCH PROBLEM

Introduction

Perinatal deaths in cattle associated with dystocia are of great economic importance to the cattle industry. Dystocias were responsible for 70 percent of all deaths at or near birth in Australia, and this amounted to 45 percent of all losses including abortion, stillbirth, and postnatal death (1). Similar figures of 60 percent and 38 percent have been reported by Patterson and Bellows (2). In all reports, most of the dystocia cases were first calf heifers. Reducing this mortality would be of substantial economic importance to the United States cattle industry.

Clenbuterol (Planipart™, NAB 365) is a highly specific, unusually long-acting (four to eight hours), sympathomimetic B_2 receptor stimulator. Its effects result in bronchodilation and tocolysis, i.e., the temporary paralysis of myometrial contractility. The tocolytic effect has been explored in the bovine and ovine for the short-term postponement of parturition to socially more acceptable hours and as an aid in resolving complicated parturitions or in obstetrical surgery. During large-scale field trials for postponement of parturition, the observation has been made by investigators that subsequent deliveries were easy and uncomplicated. Hence, an objective evaluation of the effect of Clenbuterol on the characteristics of deliveries in heifers bred to a

bull with a known high index for dystocia would be warranted. Evaluations should include: (1) potential effects of the drug on the bony and soft tissue components of the birth canal, (2) the duration of stages I, II, and III of parturition, (3) the frequency of dystocia, (4) stillbirth rate, (5) calf viability, and (6) the restoration of postpartum fertility.

Assumptions--Null Hypotheses to be Tested for the Evaluation of NAB 365

#1. No effect of the drug on dystocia rate.

#2. No effect of the drug on pelvic area.

#3. No effect of the drug on Stage I of parturition.

#4. No effect of the drug on Stage II of parturition.

#5. No effect of the drug on Stage III of parturition.

#6. No effect of the drug on calf viability.

Observations will be made on the cattle by the major investigator with the help of senior veterinary student technicians. This will place an unavoidable human error in the results based on different individuals' ability to detect cows in the first stage of parturition. Another limitation will be if the expected dystocia from the A.I. sire does not occur.

Definition of Terms

Tocolysis: The temporary paralysis of myometrial contractions.

Pelvimeter: A device used to take horizontal and vertical pelvic measurements per rectum or vagina.

Stages of Parturition: (I) Active contractions of both longitudinal and circular muscle fibers of the uterine wall and the dilation of the cervix. (II) Entrance of the fetus into the dilated birth canal, rupture of the fetal membranes, and expulsion of the fetus through the vulva. (III) Expulsion of the fetal membranes and involution of the uterus.

CHAPTER II

REVIEW OF THE LITERATURE

Introduction

The hormonal control of parturition appears to be a cascade of events, each step building on the next step, bringing about termination of pregnancy and expulsion of the fetus. The signal that initiates the cascade begins in the fetus and creates a response from the maternal endocrine system. In the bovine, much of the detail in the cascade of events lacks documented proof but, using what is known about similar species such as the goat and sheep, one can postulate the events leading to parturition.

Hormonal Control of Parturition in the Bovine

Role of the Fetus--Pituitary Adrenal Axis

The role of the fetus in timing the initiation of parturition is well accepted in most (cow, sheep, pig, and goat) domestic species (3, 4, 5, 6, 7, 8, 9, 10, 11).

The importance of an intact pituitary-adrenal axis in the fetus for initiation of parturition has been well established for cows (12, 13). Premature parturition will occur when the fetal adrenal is stimulated with corticotrophin (14, 15) and with continuous administration of dexamethasone into the fetus (14, 16). When parturition was induced by

the administration of dexamethasone to the fetal calf, the rise in estrogen and fall in progesterone in maternal circulation was similar to that seen at normal parturition (14, 16). These observations suggest that activation of the fetal pituitary-adrenal axis is responsible for the initiation of parturition in the cow (8).

The control of the fetal pituitary-adrenal axis has best been described in the sheep. In the intact fetus, cortisol production at term appears to be a result of changes in maturation of the fetal adrenal plus an increasing release of ACTH from the fetal pituitary (5, 17). The increasing release of ACTH acts on the fetal adrenal stimulating increasingly higher levels of cortisol production as the adrenal gland matures (4, 5). There is a gradual rise in the concentration of cortisol in the fetal plasma beginning 10 to 15 days prepartum, which culminates in a more rapid rise during the last two to three days of gestation (18). Similar changes in fetal cortisol levels are documented in the bovine (14, 16).

The rise in fetal cortisol acts as the signal to the dam that the fetus is ready for expulsion. Rising fetal cortisol levels also act to prepare the fetus for survival outside the uterine environment. Investigators have demonstrated the fact that glucocorticoids accelerate lung maturity by the appearance of pulmonary surfactant (19). Glucocorticoids are also known to be involved in the maturation and biochemical differentiation of other tissues in the fetus (4).

In initiating the events that lead to parturition, fetal cortisol appears to act on the placenta. The mechanism of action on the placenta by fetal cortisol in the domestic species is still under debate in the literature. In sheep, the action of rising fetal cortisol on the

placenta is well understood. Species (goat, cow, pig) which depend on the corpus luteum as the major source of progesterone have not received as much attention, and much of the action of fetal cortisol is theoretical. By using the sheep as a model and pointing out the differences in the corpus luteum dependent species, a potential picture of the action of rising fetal cortisol levels on the bovine placenta can be drawn.

In sheep, the major source of progesterone is the placenta after day 50. The increase in fetal cortisol production during the last seven to ten days of pregnancy is closely followed by a decrease in maternal progesterone during the last one to four days and by an increase in unconjugated estrogen during the last 24 hours of gestation. Evidence is accumulating that the rising fetal cortisol may be responsible for both the fall in progesterone and rise in estrogens through a direct affect on placental steroidogenesis and that this is the mechanism which influences the onset of labor (4, 20, 21).

The ovine placenta does become a complete endocrine gland capable of producing increasing amounts of estrogen in the absence of an increase in either maternal or fetal adrenal C-19 precursors (5). The placenta late in gestation secretes C-21 steroids (progesterone) and little unconjugated estrogen. In response to rising fetal cortisol concentrations, the placenta becomes capable of synthesizing estrogens from C-21 (progesterone) precursors by activating 17-alpha hydroxylase, C-17,20 lyase, and aromatase enzyme systems in the placenta. The enzyme system activity increases as fetal cortisol increases. The enzyme changes have been shown to occur <u>In Vivo</u> and <u>In Vitro</u> following spontaneous increases in endogenous fetal cortisol and/or following exogen-

ous fetal infusion of cortisol (4, 5, 6, 7, 10, 20, 21, 22). Although actual demonstration of estrogen production In Vivo has not occurred, the increased activity of the enzymes necessary for the conversion of progesterone to estrogen and demonstration In Vivo of metabolites from C-21 steroid (progesterone) reduction to C-19 steroids (estrogen) leads investigators to conclude that the pathway exists and is being utilized in the ovine (5). Other evidence which points to progesterone metabolism as a possible route for estrogen synthesis is that progesterone production remains constant while metabolism increases in late gestation (7).

The statement that fetal cortisol alters ovine placental steroidogenesis to initiate parturition may be an oversimplification. Kendall et al. (23) found that when hypophysectomized fetuses received ACTH, the normal fall in progesterone and rise in estrogen failed to occur thus implicating the hypothalmic and possibly pituitary factors in conjunction with fetal cortisol.

In the goat, cow, and sow, the predominant source of the progesterone necessary for the maintenance of pregnancy is produced by the corpus luteum. In these species, a rise in maternal estrogen and fall in maternal progesterones precedes parturition. A rise in fetal cortisol appears to trigger these maternal endocrine changes as in the sheep (14, 24).

Most of the work in the corpus luteum dependent species has been done in the goat. It has been proposed that the rising fetal cortisol levels activate enzymes in the placenta as in the sheep, leading to increased placental estrogen secretion. Flint et al. (25) showed that the increase in fetal cortisol levels preceding parturition activates the 17-alpha hydroxylase enzyme system in the caprine placenta and thus

the increasing estrogen production.

The goat's placenta is unlike the sheep in that it rapidly metabolizes progesterone to 5-beta pregnanediols In Vitro. However, like the sheep, exposure to either exogenous or endogenous glucocorticoids usually results in the formation of more polar 17-alpha hydroxylated progesterone metabolites indicating a metabolism and/or conversion similar to the fetal cortisol response in the ovine. These extracts are not formed when parturition is induced with prostaglandin which by-passes fetal cortisol increases (25). There appears to be a relatively minor activation of C-17-20 lyase enzymes in the goat as compared to the ovine (6). After exposure to glucocorticoids, the caprine placenta contains the enzymes necessary for synthesis of estrogens which are rising dramatically as fetal cortisol increases (3, 25). However, there is no In Vivo evidence for placental production of estrogens because no difference between arterial and venous levels has been detected (6, 10).

Another possible action of rising fetal cortisol is the effect it has on caprine placental lactogen. Buttle et al. (26) has identified this lactogen of placental origin in the caprine, and it has been shown to be associated with luteal support late in gestation (27). Early evidence indicates that increased fetal adrenal activity causes a decrease in caprine placental lactogen production (3).

In the bovine, fetal cortisol increases dramatically during the last ten days of gestation (14, 16). The bovine is similar to the caprine in placentation, enzyme systems, and endocrine control of parturition. Also, the placenta metabolizes progesterone in large quantities and is thought to respond to fetal cortisol similar to the goat (6).

Maternal Role in Parturition

The maternal role in parturition can be described as completing a process initiated by a signal from the fetus. The process carried out by the dam is seen as a fall in progesterone, a rise in estrogens, peaking prostaglandin levels just prior to parturition, and increases in oxytocin release just prior to and during parturition (11). Again, most of the work has been done in the caprine as an example for the bovine because of the similarities in the two species.

Luteal Support

Maintenance of the corpus luteum appears to fall into two categories in the caprine: Placental origin and pituitary origin.

Placental Origin. From the placenta, there appears to be a substance, caprine placenta lactogen (CPL) which is necessary for luteal support during pregnancy (26). Withdrawal of the source via hysterectomy during the last third of pregnancy causes abrupt declines in the circulating levels of progesterone. Progesterone levels fall to less than 50 percent of the normal prevailing levels in the intact pregnant goat (24). This suggested that hysterectomy removed luteal trophic support normally present during pregnancy. Buttle et al. (26) obtained evidence to identify and support the existence of CPL. Cowie et al. (29) demonstrated that the placental lactogen must work in conjunction with maternal pituitary factors (LH) since hypophysectomy results in abortions. A more recent study of placental lactogen in goats using radioreceptor assays has indicated that CPL was present in high concentrations throughout the latter two-thirds of pregnancy (30). The

correlation between CPL plasma changes and progesterone changes is not close, but this does not exclude a luteotrophic role for the hormone. It has been noted that CPL concentrations fell progressively during the last 15 days of pregnancy when corticosteroid concentrations were rising in fetal circulation. Similar changes, but more abrupt, were noted when corticotrophin was administered to the fetal goat raising the possibility that increasing fetal cortisol levels might inhibit the ability of the placenta to elaborate CPL (30, 31). If CPL is the placental luteotrophin in the goat, its withdrawal may act in conjunction with discrete prostaglandin F_2a (alpha) (PGF) releases to initiate luteolysis (31).

In the bovine, the structure of bovine placental lactogen has been reported (32). Initial work, however, has not indicated it to be luteotrophic (33).

Pituitary Origin. The maternal pituitary gland is also essential for luteal support in the pregnant goat. Hypophysectomy results in abortion while hypophysectomy followed by LH replacement resulted in the pregnancy being maintained (27).

Progesterone

The source of progesterone in both the caprine and bovine is the corpus luteum (8, 10). Prior to parturition, the concentration of progestagins in maternal blood plasma decreases gradually during the last 20 days of gestation and falls dramatically two to three days before parturition (34). The regression of the corpus luteum and dramatic fall of progesterone two to three days prior to parturition is the subject drawing the most interest in the literature.

Late in gestation, uterine arterial progesterone levels are greater than uterine venous progesterone levels. Thorburn (3) suggested that the placenta is using the progesterone as a precursor for estrogen synthesis. By measuring the arterial-venous progesterone difference, evidence has been presented that the gravid caprine uterus extracts significant quantities of progesterone from maternal circulation (35, 36). Thorburn and Schneider (36) showed that the arterio-venous difference in progesterone decreased as gestation increased and, in one case, the venous concentration of progesterone was actually greater than the arterial concentration. Although the number of animals was small, they suggested that in late gestation the caprine placenta produced small but increasing amounts of progesterone which would decrease the arterial-venous difference in progesterone concentration. Another possibility is that the placental uptake of progesterone is decreasing. Ainsworth and Ryan (37) and Wiener (38) have shown that In Vitro the bovine placental tissue is capable of synthesizing progesterone.

The dramatic fall in progesterone coincides with the regression of the corpus luteum which will be discussed later.

Another action of progesterone appears to be inhibition of prostaglandin release. In cycling ewes, repeated administration of exogenous progesterone resulted in an increase in prostaglandin release with each fall in progesterone. In cycling ewes, repeated administration of exogenous progesterone resulted in an increase in prostaglandin release with each fall in progesterone. The mechanism for the suppression of prostaglandin release by progesterone is unknown (31).

The effect of progesterone on the myometrium to prevent myometrial contractions appears to be mediated through controlling calcium ion

transport across the cell membrane. At the present, calcium ions are the only known stimulant which directly triggers the actinomyosin-ATP interaction and thus initiates the cyclic myometrial contractile force of labor. Progesterone promotes binding of this "activator calcium" and raises the threshhold of excitement. Prevention of the activator calcium from initiating myometrial contractions occurs even in the presence of high levels of exogenous prostaglandin which normally lowers the threshhold of excitement and allows inflow of calcium ions into the cell (39).

Estrogens

The role of estrogens in the initiation of parturition is receiving more interest in the literature. The unconjugated estrogens first appear in significant amounts in maternal plasma around days 40 to 50 of gestation in the goat. Estrogens continue to increase steadily during pregnancy before undergoing a rapid increase over the last four to five days prior to parturition (31, 40, 41, 42, 43). The estrogens present in the fetal and maternal goats are mainly estrone and estradiol 17-alpha, both considered biologically weak, and variable amounts of estradiol 17-beta (41). Flint et al. (25) has shown that estradiol 17-beta follows a similar pattern of increase as the estrone and estradiol 17-alpha prior to the dramatic fall in progesterone in normal parturition.

The source of rising estrogen concentration late in gestation is the placenta (3). Under the influence of fetal cortisol, the caprine placenta possesses the enzymes necessary for the production of estrogen. Although no actual increase of In Vivo estrogen production has been

documented, the probability cannot be dismissed that the placenta is in fact producing estrogen, possibly from progesterone precursors (3, 10). Recently, a large increase in mammary estradiol 17-beta has been demonstrated near term (44). Whether this represents De-novo synthesis or formation from placental precursors is not known (10).

The action of estrogens in the control of parturition are varied. The possibility arises that estrogens play a dual role in control of the corpus luteum. The CL of the pregnant rat is supported in part by a placental lactotropin, which may act by increasing estrogen receptors on the CL, and the increase in estrogen availability raises the LH receptor population. A similar situation may exist in the goat, in which the progressive increase in estrogen concentrations may lead to a luteotrophic complex late in gestation, since the estrogens are primarily fetal or fetoplacental in origin (5). Studies have also shown that estradiol 17-beta is luteolytic in the goat (31). The luteolytic role of estradiol 17-beta operated through prostaglandin production and release causing luteal regression. Physiological amounts of estradiol 17-beta injected into pregnant goats increased prostaglandin levels in the utero-ovarian vein and resulted in indirect luteal regression (45). The paradoxical actions of estrogens (luteolytic or luteotrophic) in the pregnant goat may well be related to the presence or absence of certain estrogen receptors in the CL. The estradiol receptor population may be regulated by caprine placental lactogen which decreases late in gestation in response to fetal cortisol (3).

The action of estradiol 17-beta causing the release of prostaglandin may come from the estradiol 17-beta traversing the placental barrier and acting on estrogen receptors in the maternal placenta which responds

by increased production of PGF. This estrogen receptor population in the placenta and endometrium may be regulated by progesterone levels (3). In the event of falling progesterone levels, the estrogen receptor population in the placenta would be increasing, amplified by itself, since estrogen is known to induce its own receptors (25).

Similar changes in estrogen levels in the cow lead to the assumptions that the action of estrogens are much the same as the goat. The major site of estrogen production is likely the placenta (14, 46). In the cow, it appears that estradiol 17-beta plays an important role in the initiation of parturition and particularly in the delivery of the placenta and membranes (8). The estrogens causing lysosomal breakdown releasing hydrolase enzymes causing dissolution of the maternal placenta and separation of the membranes (31).

Estradiol may also play an important role in oxytocin receptor population control in the placenta and myometrium (45). Roberts et al. (47) have shown that in sheep the oxytocin receptor population in the endometrium and myometrium varies during the estrous cycle and that estrogen increases their concentration.

Prostaglandins

The role of prostaglandins in pregnancy and parturition appears to be dual: (1) lysis of the corpus luteum, and (2) causing contracture of the myometrium (5).

The source of the prostaglandins appears to be the fetal membranes and maternal endometrium. In the pregnant uterus, 83 percent of the blood flow goes to the cotyledons, 3 to 4 percent to the myometrium, and 13 to 14 percent to the endometrium, suggesting that the myometrium

is not a significant source to the PGF levels. Also, In Vitro PGF production by the myometrium is only 25 percent of that produced by the maternal cotyledons (48, 49).

The role of the fetal membranes in the production of prostaglandins is not clear. Prostaglandin concentration in amnionic fluid increase during parturition and may reflect prostaglandin production of the amnion. Prostaglandins placed in the amnionic cavity can induce abortions (50, 51). Thus, speculations are drawn that prostaglandins produced in the intercotyledonary endometrium and fetal membranes may have a direct stimulating effect on the myometrium by diffusion. It is not clear if the diffusion may also be from the endometrium to the amnionic fluid (5).

The dual role of prostaglandins begins with lysis of the corpus luteum. This role is still debated in the literature. Hoffman (11) states that the prostaglandin release appears to be a coincidence of the prepartum progesterone decline rather than a cause. Other investigators believe and present evidence that the prostaglandins are indeed the primary luteolytic factor in the corpus luteum dependent species. The controversy develops around the timing of the prostaglandin release. Investigators have documented intermittent peaks of PGF release superimposed on the general increase in PGF, similar to levels observed in non-pregnant cycling ewes at the time of luteal regression (41, 54). Initial release of the prostaglandin occurred before any significant decrease in plasma progesterone levels. Further rises in PGF levels follow as progesterone levels decrease. Umo et al. (54) have suggested that the initial PGF increases occur 48 hours prior to term delivery and precedes the decrease in progesterone levels by 18 to 22 hours. Currie

and Thorburn (31) examined and found that the small amounts of PGF found in the uterine vein were luteolytic causing luteal regression and abortion.

Prostaglandin synthesis and release appears to be under the control of changes in placental progesterone and estrogen production (5). As has been previously stated, progesterone blocks prostaglandin release, and estradiol 17-beta stimulates production and release. In experiments by Liggins et al. (52), the estrogen-induced release of prostaglandin was blocked by pharmacological amounts of progesterone (200 mg./day). However, the rise in the concentration of prostaglandins in the myometrium and maternal cotyledon was not suppressed indicating that progesterone appeared to inhibit release but not the synthesis (52).

Investigators have speculated that the route by which the prostaglandins reach the myometrium is by systemic blood flow. Liggins (52) failed to demonstrate a counter-current system. During labor, when large amounts of prostaglandins are being released, significant amounts (two to three ng./ml.) were present in the jugular vein, escaping metabolism in the lung and systemic dilution (31). Although the systemic route may appear wasteful, alterations in the sensitivity of the target organs would allow low levels of prostaglandins to be effective. During the last 24 hours of gestation, the sensitivity of the myometrium increases markedly due to a decrease in plasma progesterone and an increase in estrogen levels. Thus, the low arterial levels of prostaglandin may be sufficient to exert a direct stimulation effect on the myometrium since the progesterone block is removed and the myometrium is estrogen primed (53).

The role of prostaglandin in myometrial contractions involves

calcium ion transport. PGF lowers the excitation threshhold allowing calcium ions to activate actinomyosin and ATP for contractions. The presence of progesterone blocks this effect, even in the presence of PGF by raising the threshhold for calcium transport (39).

Another role prostaglandin appears to play is relaxation of the cervix. In the presence of low progesterone levels, infusion of prostaglandins into the arterial blood supply of the uterus and cervix of sheep resulted in cervical relaxation (55).

Oxytocin

The source of oxytocin is the posterior pituitary. Oxytocin plays an important role in the second stage of labor. Distension of the vagina and cervix is known to cause release of oxytocin (56, 57). An attempt was made by Flint et al. (59) to show a relationship between PGF and oxytocin release. It was shown that after vaginal distension the oxytocin release preceded the PGF release by one minute. It has also been shown that infusions of oxytocin cause increases in PGF in the utero-ovarian vein (60).

The release of oxytocin in response to vaginal distension is enhanced by estrogen and inhibited by progesterone (61). The massive releases of PGF during labor allows some of the PGF to escape metabolism and stimulate oxytocin release in a positive feedback cascade (31, 62).

Oxytocin functions by causing an influx of the activator calcium into the cell. This activator calcium stimulates energy release from ATP for the contraction of the actinomyocin filaments (58).

The primary function of oxytocin would be to induce myometrial contractions. Contractions of the myometrium force the fetal membrane

against the softened cervix and result in cervical dilation.

Neuronal Control of Parturition

Neuronal control of the genital tract is by the autonomic nervous system. Also, there are sensitive nerve fibers in the uterine cervix and dorsal vagina that stimulate the release of oxytocin and abdominal muscular pressure during labor (63).

Autonomic nerve fibers originate in the last thoracic and first lumbar spinal cord segments and travel to the genital tract via the hypogastric nerve. Preganglionic sympathetic fibers synapse in the wall of the genital tract. Postganglionic fibers then travel to effector sites. Sympathetic nerves supply the myometrium of the genital tract, but parasympathetic nerves supply only the cervix and vagina, not the myometrium (63) and would have no effect on uterine motility.

The smooth muscle of the uterus contains two types of receptors: alpha and beta. The alpha receptors are primarily excitatory, and the beta receptors are primarily inhibitory (63). Alpha receptors have a specific affinity for norepinephrine and stimulate organ function while beta receptors have a specific affinity for epinephrine causing inhibition of organ function (58, 63). Beta receptors are found in larger numbers in uterine and respiratory tract smooth musculature (58). Storage granules of norepinephrine which are normally found at the postganglionic nerve endings are depleted during the last two-thirds of pregnancy, thus preventing local release of norepinephrine and contraction of the myometrium. However, systemic catecholamine release from the adrenal medulla (predominantly epinephrine) may cause the stimulation of the beta receptors and inhibit myometrial contractions during parturi-

tion via a systemic route rather than a neural route (63). Catecholamine release during the last third of pregnancy is primarily epinephrine, since the amount of epinephrine synthesized in the adrenal medulla is controlled by corticosteroid levels which increase the conversion of norepinephrine to epinephrine.

According to studies by Land et al. (64), there are two distinct types of beta receptors, called beta-1 and beta-2. Beta-1 receptor stimulation results in cardiac stimulation, an increase in lipolysis, and the relaxation of intestinal smooth muscle. The beta-2 receptor stimulation causes brochodilatation, relaxation of the uterus, vasodilatation, and glycolysis. These receptors have not been identified histologically but are characterized by distinct pharmacological responses.

Literature Review--Beta-2 Mimetic

Clenbuterol (Planipart™)

NAB 365

Planipart™ (4-amino-alpha tert-butyl amino methyl 35-dichlorbenzylalcohol-hydrochloride) is highly specific for beta-2 receptors and acts for long durations (58). Since its action is selective for beta-2 receptors, it has been found to be useful for the control and treatment of bronchial asthma in man and as a reliable bronchodilator in horses (58, 65). Its effects in other species include: (1) bronchodilatation in guinea pigs, cats, and dogs, (2) beta mimetic effects on the gut of guinea pigs, mice, and rats, (3) dose-related decreases in blood pressure in cats and dogs of shorter duration than the broncholytic dilation effect, and (4) In Vitro tocolysis at low doses of the rat uterus. In

Vivo tocolytic and broncholytic effects are identical (58). In comparison with other beta mimetic agents, lowering of blood pressure and the compensatory increases in heart rate are much less pronounced and of short duration with Planipart™ (66).

Studies have shown that Planipart™ acts fast and comparatively long. Zerobin and Kundig (59) have treated cattle, pigs, and sheep with Planipart™. They were attempting to delay parturition in cattle by treating at differing intervals in parturition up to and into the beginning of Stage II of parturition. Immediately after treatment, all animals calmed down with a reduced respiratory rate and spontaneous lying down. They reported an average delay time by measuring tocographic contractions of the uterus of 195 minutes; then labor was resumed with the disappearance of the drug, and deliveries did not differ from spontaneous parturition. Their subjective evaluation of Stage II was that Stage II appeared to be easier, faster, and less painful in heifers. They reported that widening of the birth canal and cervix continued during tocolysis. Offspring viability was not affected.

Parturition in sows was interrupted after one to three piglets had already been delivered. After the resumption of labor, the piglets were delivered quickly with the interval between piglets being shorter versus those recorded before treatment. No undue side effects were noted on piglet viability, placenta delivery, lactation, and maternal behavior.

Zerobin and Kundig (58) reported clinical observations in two ewes with dystocias and confirmed that Planipart™ completely suppressed the myometrial contractility for up to three hours.

Studies have shown that the tocolytic effect of Planipart™ may be reversed by oxytocin, but only after the tocolytic effect has begun to

weaken. The pharmacodynamic effect of oxytocin is mediated via specific receptors and is dependent on membrane potentials as well as the intracellular content of calcium. Beta mimetic compounds stimulate the adenylcyclase system in the cell membranes which results in an outflow of calcium from myometrial cells, causing relaxation. Oxytocin competes with the beta mimetic compounds for the control of calcium.

In non-controlled clinical studies, Ballanine et al. (67) reported on the effect of Planipart™ in clinical situations: (1) uterine prolapse (eight cases), (2) uterine hemorrhage (five cases), (3) torsion of the uterus (four cases), (4) dystocia due to fetal malposition (four cases), (5) dystocia due to fetal oversize (twelve cases), (6) dystocia due to spasm of uterine cervix (seven cases), (7) retained placenta (seven cases), and (8) preparation of caesarian section (nine cases). The recorded observations are as follows: (1) Uterine prolapse (eight cases). At the dose of 0.5 mg. parentally of NAB 365, within 30 minutes a complete reduction of uterine contractions and uterine replacement was easier. (2) Postpartum hemorrhage (five cases). Parental administration of 0.5 mg. of NAB 365 in conjunction with anticoagulation therapy reduced uterine contractions and helped stop clot expulsion. Uterine contractions which increase blood return were stopped, and clot separation and expulsion were reduced. (3) Dystocia due to uterine torsion (four cases). Reduction of the uterine contractions aided manual reduction of the torsion. (4) Dystocia due to malposition (four cases). Reduction of uterine contractions aided obstetrical manipulations, and manual delivery completed parturition. (5) Dystocia due to fetal oversize (twelve cases). In five cases, ease of fetal manipulation was

gained for manual delivery. Seven cases had caesarians, and abolition of contractions facilitated intervention. (6) Dystocia due to spasm of the uterine cervix (seven cases). Treatment before membrane rupture suppressed uterine contracture for seven to eight hours. When parturition resumed, it concluded normally in six cases. (7) Retained placenta (seven cases). Low doses were used (0.2 to 0.3 mg.) to avoid inertia when attempting to manually separate the placenta and ease powerful contractions. (8) Preparation for caesarian section. Ease of operation has been noted.

Verhülsdonk (68) used Planipart™ in a clinical trial to reduce night calving. Heifers were treated daily from the 278th day and cows daily from the 280th day (or sooner if the course of pregnancy dictated). 186 animals were used in a controlled experiment and received a placebo in the same manner. By using the beta-2 mimetic drug systemically at 9 P.M., night calving was reduced 46.8% ($p < .001$) in comparison to the controlled group. Comparisons between the two groups showed that NAB 365 worked effectively for a period of 14 hours. Another subjective observation was easier calving in first calf heifers because of better stretching of the birth canal. No effect on calf viability, Stage III of parturition or clinical appearance of the cows was noted.

Greene (69) used clenbuterol in a clinical trial to postpone parturition. In cows with a cervical diameter at treatment of less than or equal to the width of a hand. Clenbuterol delayed parturition at least 5.2 hours compared to control animals. In cows with a cervical diameter that permitted part of the fetus through the reported delay was only two hours. Clinically there was no difference in dystocia rates between treatment and control groups. Greene also used clenbuterol to reduce

night calving. 127 cows were treated at midnight if they were not showing clinical signs of parturition. Only one cow calved before 7 A.M.

CHAPTER III

MATERIALS AND METHODS

Population

Seventy-one Hereford cows were chosen for the study. Of the 71, 51 cattle were included in the trial. Two cows aborted; eight cows calved before treatment could be initiated; three cows calved too late to be included in the study; and seven cows that were initially measured for use in this study were not used. Five heifers were removed for statistical analysis. Of the 51 cattle used in the trial, 43 were first-calf heifers, and eight were second-calf heifers.

A minimum of 30 heifers thought to be settled to a Hereford bull that sired heavy birth weight calves were assigned to the study during May 1980. The remaining heifers were bred to an Angus bull of unknown progeny birth weights.

All heifers were assigned to the same pasture and nutritional regimen. They were expected to gain the approximate weight of calf and uterine fluids during the last three or four months of gestation and were expected to be in good body condition at calving.

Type of Control

The type of control was double-blind, randomized.

The 71 experimental animals were assigned to two groups in a-cor-

dance with their pelvic size (above A or below B average). Animals calved in sequence due to insemination dates (sire one first and sire two thereafter).

Heifers in groups A and B exhibiting pending parturition were assigned in numerical sequence to the treatment specified on the randomization charts, Tables I and II.

TABLE I

PLANIPART™--RANDOMIZATION OF CLINICAL SUPPLIES
GROUP A

Animal No.	Code for Treatment	Animal No.	Code for Treatment
1	I	19	I
2	T	20	D
3	O	21	T
4	D	22	L
5	P	23	T
6	W	24	F
7	A	25	P
8	H	26	O
9	C	27	H
10	F	28	I
11	L	29	W
12	K	30	C
13	W	31	F
14	H	32	D
15	C	33	L
16	O	34	K
17	K	35	A
18	A	36	P

TABLE II

PLANIPART™--RANDOMIZATION OF CLINICAL SUPPLIES
GROUP B

Animal No.	Code for Treatment	Animal No.	Code for Treatment
1	A	19	T
2	D	20	W
3	K	21	L
4	W	22	O
5	H	23	K
6	O	24	I
7	I	25	C
8	A	26	P
9	C	27	H
10	P	28	L
11	T	29	T
12	O	30	F
13	F	31	I
14	L	32	D
15	W	33	P
16	R	34	H
17	D	35	K
18	A	36	C

A placebo was utilized for control to eliminate anticipation of results. Planipart™ and placebo solutions were supplied precoded with the letters A, C, D, F, H, I, K, L, O, P, T, and W by Boehringer Ingelheim Ltd. As the study progressed, technicians recognized treated heifers on the basis of delayed parturition but, since a blind study is being conducted, they cannot anticipate results of treatment.

Dose, Route, and Duration

Single intramuscular injection of either Planipart™ 0.8 mcg./kg. (0.0267 ml./kg. of solution containing 30 mcg./ml.) or placebo 0.0267 ml./kg. were used.

Method of Evaluation

Pelvic Areas

Pelvic areas were taken at approximately seven months of gestation. This was done per rectum taking vertical and horizontal diameters and multiplying these for a relative pelvic area (70). Heifers were stratified into the top 50 percent and lower 50 percent areas for assignment to treatment groups. Pelves were also measured at prepartum treatment and immediately postpartum.

Observation of Parturition and Treatment

Heifers were observed during the last ten days of gestation three times daily for pending parturition. When parturition was imminent (distended udder, relaxed sacrosiatic ligaments, release of cervical plug), they were observed every two hours for signs of the first stages

of labor. This was confirmed by vaginal examination. If the cervix was dilated five centimeters or more, the heifer was treated with placebo or Planipart™ intramuscularly. Stage of labor, including cervical dilation at treatment, and the times of the above episodes were recorded.

The heifers were placed in a pen to continue parturition. The second stage of labor (abdominal press and presentation of fetal membranes--water sac at the vulva) was the next benchmark event. To standardize the observations for the beginning of the second stage of parturition, the landmark used to give consistency among the observers was the presence of fetal membrane visible in the vulva. However, observations were recorded a minimum of every two hours from treatment to parturition.

Once the water sac was observed at the vulva, two hours could pass before the heifer was examined for dystocia. At this time, whatever assistance was required to deliver a live calf was used. Dystocia scores were: (1) normal parturition, (2) token assistance of one person by hand (no mechanical assistance), (3) mechanical assistance required, but not dangerous to heifer or calf, (4) caesarian or equivalent assistance required, and (5) malpresentation, position or posture.

Calf viability was scored as alive or dead at birth, also, time from birth to nursing was recorded. If a calf did not nurse within two hours of birth, it was fed colostrum via stomach tube and recorded. Climatic conditions were recorded at birth. Calf weight was recorded within 12 hours after birth and every two weeks until eight weeks of age.

Time of delivery of the placenta was recorded. Placentas were considered to be retained if not delivered within eight hours of parturition.

Evaluation of Postpartum Ovarian Luteal Functions

Weekly uterine palpations were conducted through 40 days post-partum to evaluate uterine involution. The parameters recorded were cervical diameter and horn diameters in millimeters.

CHAPTER IV

DESCRIPTION OF THE DATA ANALYSIS

Analysis of the data was done based on the following null hypothesis: (1) no effect of the drug on dystocia rate, (2) no effect of the drug on pelvic area, (3) no effect of the drug on length of Stage I of parturition, (4) no effect of the drug on length of Stage II of parturition, (5) no effect of the drug on Stage III of parturition and (6) no effect of the drug on calf viability.

Clinical observations were used to test the drug effect on Stage III, dystocia rate, and calf viability. Statistical analysis of the clinical data was used to test the remaining null hypotheses. The null hypothesis assumes that the treatment and the controls are equal (no effect due to treatment). A statistical analysis was done in order to test the null hypothesis. The experiment done was one with two unequal-sized groups, randomized selection, and independent samples. Where the variances in each group were judged to be equal, a standard t test was done on the observed mean difference between the treatment and the control groups in order to test the null hypothesis. A pooled estimate of the variance follows the formula:

$$s_p^2 = \frac{\Sigma y_A^2 + \Sigma y_B^2}{(n_A - 1) + (n_B - 1)}$$

where n equals the number of observations.

The student t test on the observed mean difference was then computed using the following formula:

$$s_{\bar{y}_A - \bar{y}_B} = \sqrt{\frac{s_p^2}{n_A} + \frac{s_p^2}{n_B}}$$

$$t \text{ calc} = \frac{|\bar{y}_A - \bar{y}_B|}{s_{\bar{y}_A - \bar{y}_B}}$$

Where the variances were judged to be unequal, then a t'test where $t' \text{ calc} = (\bar{x}_1 - \bar{x}_2)/ \sqrt{s_1^2/n_1 + s_2^2/n_2}$ was used instead of a standard t test.

The observed significance level of 2.5 percent in the data indicates that if all the treatments were equal (the treatment results would equal control results) and the samples were taken at random, we could expect to see results similar to the one we have less than 2.5 percent of the time due to sampling of the population. Therefore, we have evidence to reject the null hypothesis.

TABLE III

GROUP A--LENGTH OF STAGE I (MINS)

Treatment	Control
135	180
180	55
420	30
1350	195
105	60
960	110
255	150
510	230
760	150
480	30
75	
510	
340	

$\Sigma y_t = 6080.00$ $\quad$ $\Sigma y_c = 1190.00$

$n = 13$ $\quad$ $n = 10$

$\bar{y}_t = 467.69$ $\quad$ $\bar{y}_c = 119.00$

$s_{y_t} = 371.15$ $\quad$ $s_{y_c} = 72.44$

$\Sigma y^2_t = 1653030.76$ $\quad$ $\Sigma y^2_c = 47240.00$

$s^2_{y_t} = 137752.5641$ $\quad$ $s^2_{y_c} = 5248.8889$

$$F \text{ calc} = \frac{137752.5641}{5248.8889} = 26.24$$

DF 12 & 9

F tab = 3.87

$$s^2_{\bar{y}_t - \bar{y}_c} = \frac{s_t^2}{n_t} + \frac{s_c^2}{n_c} = \frac{137752.56}{13} + \frac{5248.88}{10} = 19596.35 + 924.89$$

$$= 11121.24$$

TABLE III (Continued)

$$s_{\bar{y}_t - \bar{y}_c} = \sqrt{11121.24} = 105.45$$

$$t'calc = \frac{|\bar{y}_t - \bar{y}_c|}{s_{\bar{y}_t - \bar{y}_c}} = \frac{467.69 - 119.00}{105.45} = \frac{348.69}{105.45} = 3.306$$

$$p < 0.025$$

TABLE IV

GROUP B--LENGTH OF STAGE I (MINS)

Treatment	Control
150	30
195	185
75	210
165	60
900	330
720	120
550	105
375	135
465	85
555	100
165	
30	
615	
$\Sigma y_t = 4960.00$	$\Sigma y_c = 1360.00$
$n = 13$	$n = 10$
$\bar{y}_t = 381.54$	$\bar{y}_c = 130.00$
$s_{y_t} = 274.33$	$s_{y_c} = 86.69$
$s^2_{y_t} = 75255.77$	$s^2_{y_c} = 7515.56$

$$F \text{ calc} = \frac{75255.77}{7515.56} = 10.01$$

DF 12 & 9

F tab = 3.87

$$s^2_{\bar{y}_t - \bar{y}_c} = \frac{s_t^2}{} + \frac{s_c^2}{} = \frac{75255.77}{13} + \frac{7515.56}{10} = 5788.91 + 751.56$$

$$= 6540.47$$

TABLE IV (Continued)

$$s_{\bar{y}_t - \bar{y}_c} = \sqrt{6540.47} = 80.87$$

$$t'calc = \frac{|\bar{y}_t - \bar{y}_c|}{s_{\bar{y}_t - \bar{y}_c}} = \frac{381.59 - 130.00}{80.87} = \frac{251.54}{80.87} = 3.11$$

$$p < 0.025$$

TABLE V

GROUP A--LENGTH OF STAGE II (MINS)

Treatment	Control
90	20
20	50
65	35
30	75
45	120
15	55
145	95
60	145
45	150
75	120
60	
15	
35	
$\Sigma y_t = 700.00$	$\Sigma y_c = 865.00$
$n = 13$	$n = 10$
$\bar{y}_t = 53.85$	$\bar{y}_c = 86.50$
$s_{y_t} = 35.95$	$s_{y_c} = 46.31$
$\Sigma y^2{}_t = 15507.67$	$\Sigma y^2{}_c = 19302.50$
$s^2_{y_t} = 1292.31$	$s^2_{y_c} = 2144.72$

$$F \text{ calc} = \frac{2144.72}{1292.31} = 1.66$$

$$F \text{ tab} = 3.87$$

$$DF \ 12 \ \& \ 9$$

$$sp^2 = \frac{\Sigma y^2{}_t + \Sigma y^2{}_c}{(n_t - 1) + (n_c - 1)} = \frac{15507.69 + 19302.50}{21} = \frac{34810.19}{21} = 1657.63$$

TABLE V (Continued)

$$s^2_{\bar{y}_t - \bar{y}_c} = \frac{sp^2}{n_t} + \frac{sp^2}{n_c} = \frac{1657.63}{13} + \frac{1657.63}{10} = 127.51 + 165.77 = 293.28$$

$$s_{\bar{y}_t - \bar{y}_c} = \sqrt{293.28} = 17.13$$

$$\text{calc } t = \frac{|\bar{y}_t - \bar{y}_c|}{s_{\bar{y}_t - \bar{y}_c}} = \frac{53.85 - 86.50}{17.13} = \frac{32.65}{17.13} = 1.91$$

TABLE VI

GROUP B--LENGTH OF STAGE II (MINS)

Treatment	Control
180	105
105	160
225	150
90	125
95	125
240	70
40	45
5	270
25	60
150	85
130	
90	
20	

$\Sigma y_t = 1395.00$ $\quad$ $\Sigma y_c = 1195.00$

$n = 13$ $\quad$ $n = 10$

$\bar{y}_t = 107.31$ $\quad$ $\bar{y}_c = 119.50$

$s_{y_t} = 75.96$ $\quad$ $s_{y_c} = 65.17$

$s^2_{y_t} = 5769.23$ $\quad$ $s^2_{y_c} = 4246.94$

$$F \text{ calc} = \frac{5769.23}{4246.94} = 1.36$$

$$F \text{ tab} = 3.87$$

$$sp^2 = \frac{\Sigma_{y_t} + \Sigma_{y_c}}{(n_t - 1) + (n_c - 1)} = \frac{69230.77 + 38222.50}{21} = \frac{107453.27}{21} = 5116.82$$

TABLE VI (Continued)

$$s^2_{\bar{y}_t - \bar{y}_c} = \frac{sp^2}{n_t} + \frac{sp^2}{n_c} = \frac{5116.82}{13} + \frac{5116.82}{10} = 393.60 + 511.68 = 905.28$$

$$s_{\bar{y}_t - \bar{y}_c} = \sqrt{905.28} = 30.09$$

$$\text{calc } t = \frac{|\bar{y}_t - \bar{y}_c|}{s_{\bar{y}_t - \bar{y}_c}} = \frac{107.31 - 119.50}{30.09} = 0.41$$

TABLE VII

GROUP A--PELVIC AREA AT BIRTH (cm^2)

Treatment	Control
276	285
300	300
300	304
310	285
283	272
300	287
300	285
292	285
280	304
308	394
261	
252	
305	
$\Sigma y_t = 3767.00$	$\Sigma y_c = 2901.00$
$n = 13$	$n = 10$
$\bar{y}_t = 289.77$	$\bar{y}_c = 290.10$
$s_{y_t} = 18.22$	$s_{y_c} = 10.22$
$\Sigma y^2_t = 3982.31$	$\Sigma y^2_c = 940.90$
$s^2_{y_t} = 331.86$	$s^2_{y_c} = 104.54$

$$F \text{ calc} = \frac{331.86}{104.54} = 3.17$$

DF 12 & 9

F tab = 3.87

$$sp^2 = \frac{\Sigma y^2_t + \Sigma y^2_c}{(n_t - 1) + (n_c - 1)} = \frac{3982.31 + 940.90}{21} = 234.44$$

TABLE VII (Continued)

$$s^2_{\bar{y}_t - \bar{y}_c} = \frac{sp^2}{n_t} + \frac{sp^2}{n_c} = \frac{234.44}{13} + \frac{234.44}{10} = 18.04 + 23.44 = 41.47$$

$$s_{\bar{y}_t - \bar{y}_c} = \sqrt{41.47} = 6.44$$

$$\text{calc } t = \frac{|\bar{y}_t - \bar{y}_c|}{s_{\bar{y}_t - \bar{y}_c}} = \frac{289.77 - 290.10}{6.44} = \frac{0.33}{6.44} = 0.051$$

TABLE VIII

GROUP B--PELVIC AREA AT BIRTH (cm^2)

Treatment	Control
259	240
261	283
270	285
203	294
238	292
276	252
279	245
261	300
280	230
277	266
245	
285	
304	
$\Sigma y_t = 3428.00$	$\Sigma y_c = 2687.00$
$n = 13$	$n = 10$
$\bar{y}_t = 264.46$	$\bar{y}_c = 268.70$
$s_{y_t} = 25.28$	$s_{y_c} = 25.39$
$\Sigma y^2{}_t = 7669.23$	$\Sigma y^2{}_c = 5802.10$
$s^2_{y_t} = 639.10$	$s^2_{y_c} = 644.68$

$$F \text{ calc} = \frac{639.10}{644.68} = 1.01$$

DF 12 & 9

F tab = 3.87

$$sp^2 = \frac{\Sigma y^2{}_t + \Sigma y^2{}_c}{(n_t - 1) + (n_c - 1)} = \frac{7669.23 + 5802.10}{21} = \frac{13471.33}{21} = 641.49$$

TABLE VIII (Continued)

$$s^2_{\bar{y}_t - \bar{y}_c} = \frac{sp^2}{n_t} + \frac{sp^2}{n_c} = \frac{641.49}{13} + \frac{641.49}{10} = 49.35 + 64.15 = 113.50$$

$$s_{\bar{y}_t - \bar{y}_c} = \sqrt{113.50} = 10.65$$

$$\text{calc } t = \frac{|\bar{y}_t - \bar{y}_c|}{s_{\bar{y}_t - \bar{y}_c}} = \frac{264.46 - 268.70}{10.65} = \frac{4.24}{10.65} = 0.40$$

TABLE IX

LENGTH OF STAGE II IN CONTROL HEIFERS VS
LENGTH OF STAGE II IN TREATMENT HEIFERS
WITH CERVICAL DIAMETER > 10 cm.
AT TREATMENT, (MINS)

Treatment > 10 cm.	Control
135	180
180	55
420	30
105	195
255	60
510	110
75	150
340	230
30	150
85	30
150	30
75	185
165	210
465	60
165	330
30	120
	105
	135
	85
	100

$\Sigma y_t = 3185.00$ $\quad$ $\Sigma y_c = 2550.00$

$n = 16$ $\quad$ $n = 20$

$\bar{y}_t = 199.06$ $\quad$ $\bar{y}_c = 127.50$

$\Sigma y^2_t = 992225.00$ $\quad$ $\Sigma y^2_c = 441450.00$

$(\Sigma y)^2/n = 634014.06$ $\quad$ $(\Sigma y)^2/n = 325125.00$

$\Sigma y^2_t = 358210.94$ $\quad$ $\Sigma y^2_c = 116325.00$

$s^2_{y_t} = 23880.73$ $\quad$ $s^2_{y_c} = 5816.25$

$$F \text{ calc} = \frac{23880.73}{5816.25} = 4.11$$

DF 19 & 15

F tab = 2.76

TABLE IX (Continued)

$$s^2_{\bar{y}_t - \bar{y}_c} = \frac{s^2_{\bar{y}_t}}{n_t} + \frac{s^2_{\bar{y}_c}}{n_c} = \frac{23880.73}{16} + \frac{5816.25}{20} = 1492.55 + 290.81$$

$$= 1783.36$$

$$s_{\bar{y}_t - \bar{y}_c} = \sqrt{1783.36} = 42.23$$

$$t'calc = \frac{|\bar{y}_t - \bar{y}_c|}{s_{\bar{y}_t - \bar{y}_c}} = \frac{199.06 - 127.50}{42.23} = \frac{71.56}{42.23} = 1.69$$

TABLE X

LENGTH OF STAGE II FOR HEIFERS CALVING LESS THAN 2 HOURS AND THOSE CALVING GREATER THAN OR EQUAL TO 2 HOURS (MINS)

< 2 Hours	≥ 2 Hours
90	145
20	120
65	145
30	150
45	120
15	180
60	225
45	240
75	150
60	130
15	160
35	150
20	125
50	125
35	270
75	
55	
95	
105	
90	
95	
40	
5	
25	
90	
20	
105	
70	
45	
60	
85	
1720.00	2435.00
$n = 31$	$n = 15$
$\bar{y} = 55.48$	$\bar{y} = 162.33$
$Sy_t = 29.56$	$Sy_c = 46.56$

TABLE X (Continued)

$$\Sigma y^2_t = 26217.74 \qquad \Sigma y^2_c = 30343.33$$

$$Sy^2_t = 845.73 \qquad Sy^2_c = 2022.89$$

$$Sp^2 = \frac{26217.74 + 30343.33}{30 + 14} = 1285.48$$

$$S^2_{\bar{y}_t - \bar{y}_c} = \frac{Sp^2}{n_t} + \frac{Sp^2}{n_c} = \frac{1285.48}{31} + \frac{1285.48}{15} = 41.47 + 85.70 = 127.17$$

$$S_{\bar{y}_t - \bar{y}_c} = \sqrt{127.17} = 11.28$$

$$\text{Calc } t = \frac{|\bar{y}_t - \bar{y}_c|}{S_{\bar{y}_t - \bar{y}_c}} = \frac{|55.48 - 162.33|}{11.28} = 9.48$$

$$p < 0.001$$

TABLE XI

LENGTH OF STAGE II BETWEEN GROUPS (MINS)

A	B
90	180
20	105
65	225
30	90
45	95
15	240
145	40
60	5
45	25
75	150
60	130
15	90
35	20
20	105
50	160
35	150
75	125
120	125
55	70
95	45
145	270
150	60
120	85
2590.00	1565.00
n = 23	n = 23
$\bar{y}$ = 112.61	$\bar{y}$ = 68.04
Sy = 70.16	Sy = 43.08

TABLE XI (Continued)

$\Sigma y^2 = 108293.48$ $\Sigma y^2 = 40836.96$

$Sy^2 = 4922.43$ $Sy^2 = 1856.23$

$$Sp^2 = \frac{\Sigma y^2_A + \Sigma y^2_B}{2(n - 1)} = \frac{108293.48 + 40836.96}{44} = 3389.33$$

$$S\bar{y}_A - \bar{y}_B = \sqrt{\frac{2(Sp^2)}{22}} = \sqrt{308.12} = 17.55$$

$$\text{Calc } t = \frac{|\bar{y}_A - \bar{y}_B|}{S\bar{y}_A - \bar{y}_B} = \frac{112.61 - 68.04}{17.55} = \frac{44.57}{17.55} = 2.54$$

tab t = 44 DF

$p < 0.025$

CHAPTER V

RESULTS AND DISCUSSION

Limitations and Error

Observations for pending signs of parturition and signs indicating that Stage I had begun were made by senior veterinary students and the principal investigators. This placed an error on the experiment dependent on the observers' ability to detect the subtle or non-existent signs of Stage I. There was a wide range in ability among the students to detect early Stage I. Some students seemed to never miss while others could only find cows in Stage I by luck. Therefore, several cows which should have been detected were missed completely, and others which were detected late in Stage I should have been detected earlier. This is one reason for the wide range in the variances for Stage I data.

The most consistent method we could use to find cows in Stage I with the cervix less than fully dilated was to check the sacrosciatic ligament on all cows twice daily, spend all spare time watching the cows for subtle changes in their attitude or habits, and doing vaginal examination on cows with loose sacrosciatic ligaments every four to six hours. On more than one occasion, cows calved without showing any impending signs of parturition (i.e., udder filling, cervical plug, loose sacrosciatic) for a detectable length of time.

The major factor which caused the wide range in the variances for Stage I data was the extent of cervical dilation at treatment. For

those cows with cervical dilation of greater than ten centimeters, the effect of treatment was negligible. An analysis by a t' test showed no significant mean difference at the 5% level between the treatment animals with greater-than-ten-centimeter cervical dilation at treatment versus the control animals.

TABLE XII

LENGTH OF STAGE II IN CONTROL HEIFERS VS LENGTH OF STAGE II IN TREATMENT HEIFERS WITH CERVICAL DIAMETER > 10cm AT TREATMENT. (MINS)

Treatment > 10 cm.	Control
$\Sigma y_t = 3185.00$	$\Sigma y_c = 2550.00$
$n = 16$	$n = 20$
$\bar{y}_t = 199.06$	$\bar{y}_c = 127.50$
DF 19 & 15	

The relationship between cervical diameter at treatment and length of delay can be seen in the graphs 1 and 2 (see appendix).

Another problem which contributed error and the variance difference was a very poor A.I. conception. Therefore, we were not able to place any faith on the breeding dates and predicting parturition.

Also, the expected dystocias from the Hereford bull did not occur. Of the 46 cows used, 35 calved with no assistance. Of the 11 requiring assistance, two were due to malposture or position, and the remaining nine were all soft tissue restructions, i.e., vulva, hymen. No animal required assistance because of pelvic restriction. Therefore, evaluation of this drug to alleviate or lessen dystocias in heifers could not be done.

Shortly after the trial began, we were notified that the expiration date on the placebo had expired and could be precipitating. It was forming a precipitate and was removed from use, and a saline placebo was used. This eliminated the blind assessment and may have contributed to some bias.

Pelvic Area

Pelvic area of the dam has been shown to have a high correlation with dystocia with heavy birth weights increasing the correlation (70). The trial was designed to use this multiple correlation in testing the effect of the drug but, as previously stated, the expected dystocias due to high birth weight did not occur. This left only the effect of the drug on pelvic area to be evaluated. The idea was postulated that increased time in Stage I could allow the soft tissue attachments of the pelvis to continue to relax, thus increasing their stretch during Stage II and allowing a larger pelvic area.

By measuring the pelvic area with the pelvimeter at treatment and then immediately postpartum, a significant increase in the stretching of the soft tissue attachments yielding an increased pelvic area should be detected. Measurements during this trial showed no statistically sig-

nificant difference between treatment and control pelvic areas for either group.

Schebitz (71) showed that the pelvic lumen was greatest during abdominal contractions in lateral recumbancy and indicated that pelvic lumen increases may be a dynamic one, i.e., during an abdominal contraction.

This would point to the possibility of the drug effect being a dynamic one and not detectable if measured just before and after parturition. If there was an increased dynamic effect of the drug due to an increased relaxation of the soft tissue pelvic attachments, then abdominal contractions should have caused an increase in the pelvic lumen. If this effect were present, then Stage II for the treatment group should have been easier, therefore shorter. Since no significant difference was found in Stage II length, then this increase in dynamic effect for the treatment group probably does not exist.

TABLE XIII

PELVIC AREA AT BIRTH--GROUP A (cm^2)

Treatment	Control
$\Sigma y_t = 3767.00$	$\Sigma y_c = 2901.00$
$n = 13$	$n = 10$
$\bar{y}_t = 289.77$	$\bar{y}_c = 290.10$

TABLE XIV

PELVIC AREA AT BIRTH--GROUP B (cm^2)

Treatment	Control
$\Sigma y_t = 3408.00$	$\Sigma y_c = 2687.00$
$n = 13$	$n = 10$
$\bar{y}_t = 264.46$	$\bar{y}_c = 268.70$

Stage I Delay

The length of Stage I was greatly increased due to the treatment. The mean difference for both groups A and B was significant at the 0.025 level.

TABLE XV

STAGE I--GROUP A (MINS)

Treatment	Control
$\Sigma y_t = 6080.00$	$\Sigma y_c = 1190.00$
$n = 13$	$n = 10$
$\bar{y}_t = 467.69$	$\bar{y}_c = 119.00$
Range = 75-1350	Range = 30-230
$p < 0.025$	DF 21

TABLE XVI

STAGE I--GROUP B (MINS)

Treatment	Controls
$\Sigma y_t = 4960.00$	$\Sigma y_c = 1360.00$
$n = 13$	$n = 10$
$\bar{y}_t = 381.54$	$\bar{y}_c = 130.00$
Range = 30-900	Range = 30-330
$p < 0.025$	DF 21

There is evidence against the null hypothesis of no effect of the drug on the length of Stage I.

The variance within each group used in the analysis was very large making a detailed analysis of this very cumbersome and difficult. This variance was caused primarily by the ability of the observer to detect cows early in Stage I and individual cow variation. As graphs 1 and 2 illustrated, length of delay is shortened as cervical diameter at treatment increased.

As already shown the drug had no delay on parturition after the cervix reached 10 cm or greater in dilation. At this stage of dilation fetal membranes and appendages are protruding through the cervix stimulating abdominal contractions. This demonstrates the ineffectiveness of tocolysis in competition with stimuli for abdominal contractions.

STAGE II

The length and, therefore, the ease of Stage II was not affected by treatment. The mean difference for either group, A or B was not significant at the 0.025 level.

TABLE XVII

STAGE II--GROUP A (MINS)

Treatment	Control
$\Sigma y_t = 700.00$	$\Sigma y_c = 865.00$
$n = 13$	$n = 10$
$\bar{y}_t = 53.85$	$\bar{y}_c = 86.50$
Range = 15-145	Range = 20-150

TABLE XVIII

STAGE II--GROUP B (MINS)

Treatment	Control
$\Sigma y_t = 1395.00$	$\Sigma y_c = 1195.00$
$n = 13$	$n = 10$
$\bar{y}_t = 107.31$	$\bar{y}_c = 119.50$
Range = 5-240	Range = 45-270

Since there was no detectable affect of the drug on Stage II the animals were grouped according to pelvic areas irrespective of treatment and controls. This was done to evaluate the length of Stage II for those animals with large pelvic areas vs. those with small pelvic areas. The mean difference between groups (A- $\bar{y}$ = 68.04; B- $\bar{y}$ = 112.61) was significant at the .025 level.

TABLE XIX

LENGTH OF STAGE II BETWEEN GROUPS (MINS)

A	B
$\Sigma y_A = 1565.00$	$\Sigma y_B = 2590.00$
$n = 23$	$n = 23$
$\bar{y}_A = 68.04$	$\bar{y}_B = 112.61$

In this study of the 15 animals requiring some assistance with birth 6 were in group A and 10 were in group B. The significant mean difference between groups indicated that pelvic area had more effect on the length and ease of Stage II then did the treatment.

Stage II: Less Than Two Hours, Greater Than or Equal to Two Hours

Since there was no affect on Stage II due to treatment, we wanted to look at the length of Stage II for those cows calving within the 2 hour limit. The mean difference for those < 2 hours was $\bar{y}$ = 55.48 minutes and those $\geq$ 2 hours was $\bar{y}$ = 162.33.

TABLE XX

LENGTH OF STAGE II FOR HEIFERS CALVING LESS THAN 2 HOURS AND THOSE CALVING GREATER THAN OR EQUAL TO 2 HOURS (MINS)

< 2 Hours	$\geq$ 2 Hours
Σy = 1720.00	Σy = 2435.00
n = 31	n = 15
$\bar{y}$ = 55.48	$\bar{y}$ = 162.33
p < .001	
DF = 30 & 14	

Due to the protocol of this trial those cows going over 2 hours and not about to finish Stage II were assisted. This would shorten the true length of Stage II for those going over 2 hours. In the group $\geq$ hours (n = 15) only 4 were unassisted. The mean difference was significant at the .001 level. The mean value of 55.48 minutes for the less than 2 hour group was shorter than expected. This indi-

cates that calving assistance may be warranted sooner than presently thought. Additional data needs to be evaluated to substantiate this observation.

Stage III

By definition, Stage III includes both the shedding of the fetal membrane and uterine involution. Only one cow failed to shed her membrane. Delay of Stage I had no effect on the shedding of the fetal membrane.

Uterine involution was evaluated by weekly rectal palpation through 40 days postpartum. There was no palpable difference in the uterine involution for the treatment and control groups.

Calf Viability

There was no effect on calf viability due to treatment. One calf was born dead in group A treatment, but no other calves showed any effects from Stage I delay. One cow was delayed for 22 hours, and her calf was up and nursing well within the two-hour limit.

When you look at groups irrespective of treatment; group A, 2 calves did not nurse within the 2 hour limit and in group B, 7 calves did not nurse within the 2 hour limit and required assistance. With the large main difference for the length of Stage II between groups, the smaller pelvic area appeared to affect calf viability.

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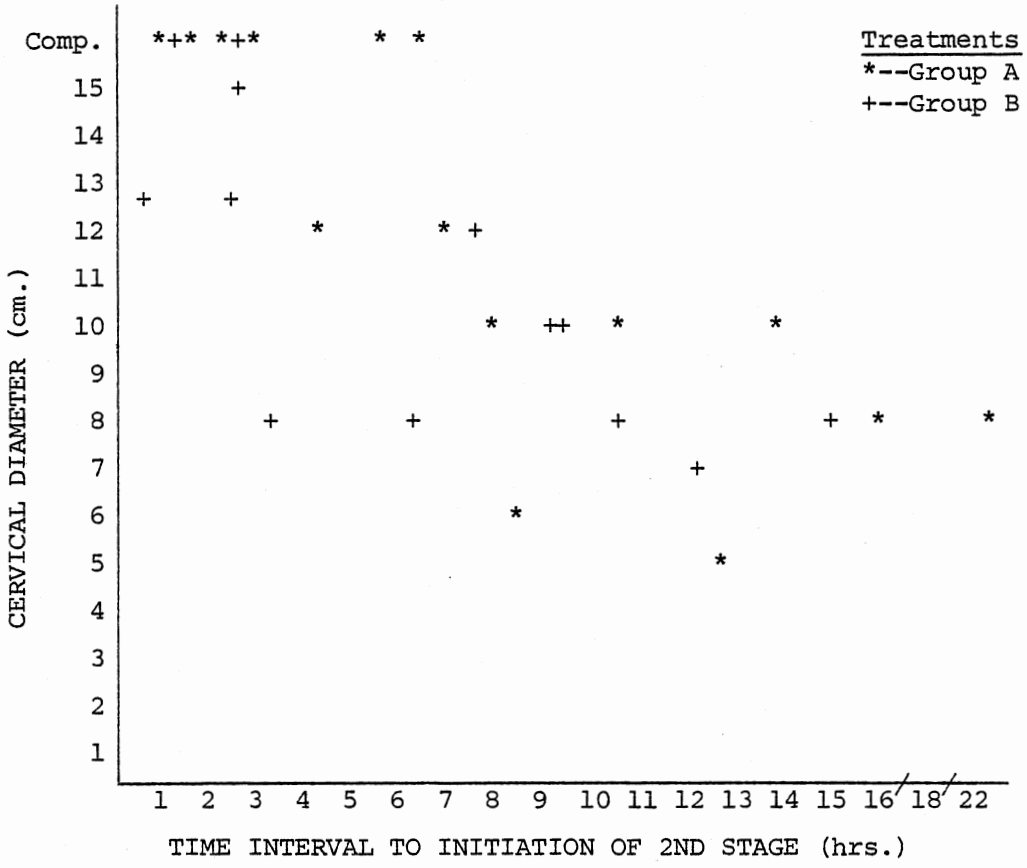
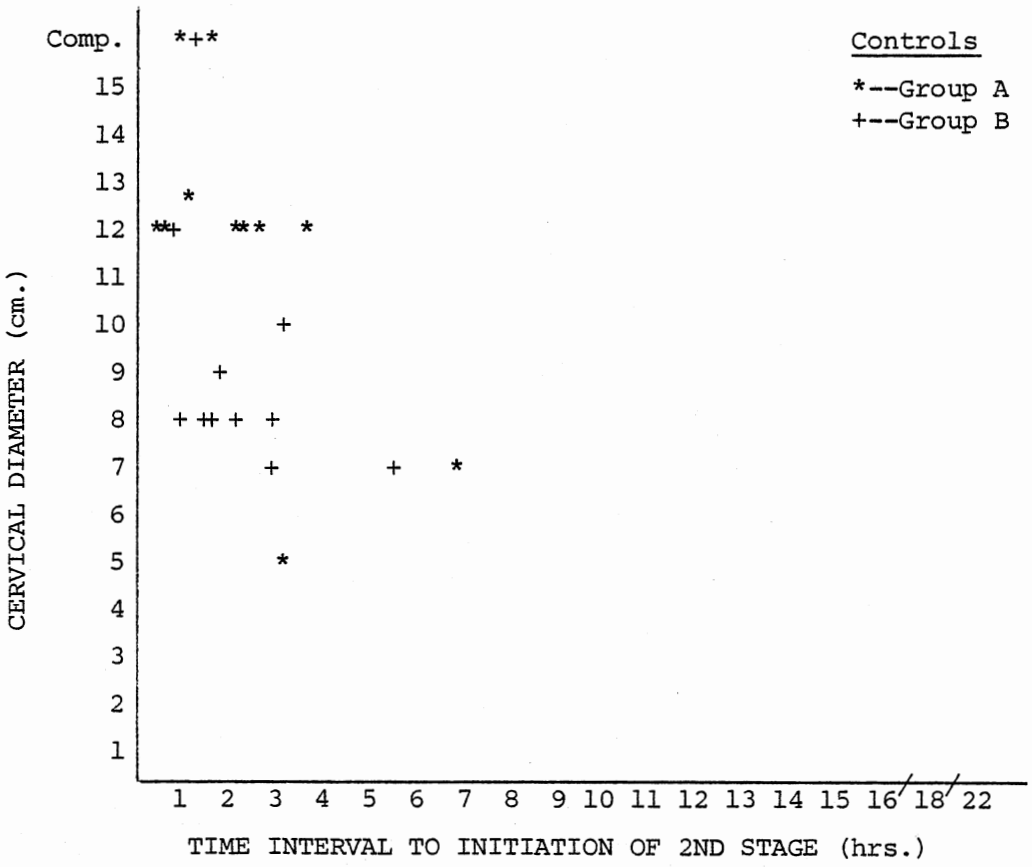
APPENDIX A

NAB 365 TREATMENTS



APPENDIX B

CONTROLS



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Master of Science

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