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THE INCIDENCE OF MULTIPLE OVULATION
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STANDARDBRED FARM

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David Thomas Cross

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Richard J. Dunn
Major professor

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# THE INCIDENCE OF MULTIPLE OVULATION AND TWINNING ON A MICHIGAN STANDARDBRED FARM

By

David Thomas Cross

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ABSTRACT

THE INCIDENCE OF MULTIPLE OVULATION AND TWINNING ON A MICHIGAN STANDARDBRED FARM

By

David Thomas Cross

The present study involved a review of breeding records from a Michigan Standardbred farm for the years 1980 through 1984. Objectives were to determine the incidence of multiple ovulation and twinning in a population of Standardbred mares, to study the effect of reproductive status and age group on multiple ovulation, twinning and abortion, and to determine the effect of human chorionic gonadotropin administration on multiple ovulation.

An effect of reproductive status on follicle number was found, but reproductive status had no effect on ovulation rate. Age group had no effect on follicle number or ovulation rate. No conclusions could be made about the effect of time interval between double ovulations on the number of embryos. Reproductive status had no effect on abortion rate. Abortion rate was dependent on age group, but none of the specific comparisons made were significant. Ovulation rate was independent of hCG dosage.

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# INTRODUCTION

Of the domestic livestock species, horses have the lowest conception rates. This is partially due to the arbitrary January first birthdate imposed by most registries. Maximum ovarian function in the mare occurs during the months of increasing photoperiod, but due to the industry pressures for foals born early in the year, breeders often begin breeding in February and March when many mares are in winter anestrous. Of the mares conceiving during a breeding season, a number can be expected to experience a pregnancy loss resulting in economic losses for breeding fees, board bills and loss of a marketable product. Twinning can be the single most important cause of abortions, and therefore preventative management practices are usually followed.

The incidence of multiple ovulation and twinning has been investigated for several horse breeds, but the concentration of studies has been on Thoroughbreds. The majority of these studies are based upon the collection and analysis of survey data. Recently, research has moved in the direction of ultrasonic detection of early twin pregnancies.

The objectives of this study were to determine the incidence of multiple ovulation and twinning in a population of Michigan Standardbred mares, to study the effect of

reproductive status and age group on multiple ovulation, twinning and abortion, and to determine the effect of human chorionic gonadotropin administration on multiple ovulation.

# REVIEW OF LITERATURE

Twinning has been reported to be the largest single cause of equine abortions, ranging from 22% to 29% (Platt, 1973; Whitwell, 1980). The incidence of twinning is highest in Thoroughbreds and lowest in ponies (Blakeslee and Hudson, 1942; Ginther, 1979). Survival rates of twins are higher in draft horse breeds, which is probably due to the greater uterine capacity (Blakeslee and Hudson, 1942; Vandeplascche et al., 1970; Jeffcott and Whitwell, 1973). With the exception of one case of identical twins reported in the literature (Jeffcott and Whitwell, 1973), all twins of the same sex have proven to be dizygotic, based on differences in hair and hoof markings and blood groups (Vandeplassche et al., 1970; Podliachouk et al., 1974).

## Multiple Ovulation and Twinning

The incidence of multiple ovulation varies from breed to breed and between farms within the same breed (Ginther et al., 1982a). Ginther (1979) reported that the incidence of multiple ovulation in various horse breeds ranged from 4% to 43%, with an average of 16%. Overall, the highest rate of multiple ovulation is found in the Thoroughbred, which also has the highest rate of twinning (Ginther et al., 1982a). The incidence of multiple ovulation is very low in ponies as is the incidence of twinning (Jeffcott and

Whitwell, 1973). Other contributing factors in addition to breed that have been implicated in multiple ovulation and twinning include heritability, reproductive status, mare age, increased daylength and a high plane of nutrition (Vandeplassche et al., 1970; Whitwell, 1980; Ginther, 1982, Urwin and Allen, 1983).

The results of a survey of veterinarians by Ginther et al. (1982a) indicated that the increase of multiple ovulation and twin pregnancy is higher in some individuals and family lines. Examination of breeding records by the same group support this view. These authors suggested that this would account for differences in twinning rates between farms within the same breed. Blakeslee and Hudson (1942) referred to a mare with three sets of twins. A daughter of, and a half-sister to, this mare each had a set of twins. Butz and Schmahlsteig (1953) stated that the natural tendency toward twinning is hereditary and Platt (1973) reported differences between stallions in recorded frequency of twinning.

Results from several studies have indicated that there is a relationship between reproductive status, the incidence of multiple ovulation and twinning. Ginther et al. (1982a) reported the incidence of multiple ovulation and twinning to be lower in foaling mares than in barren or maiden mares. Ginther, in 1983, found that double ovulations and twins were diagnosed more frequently in barren mares than in lactating mares (11% and 6% versus 5% and 1%). Similar results were reported by Deskur (1985). A

suppressive effect of nursing on reproductive function is thought to be involved (Ginther, 1979; Ginther et al., 1982a; Ginther, 1983). Studies reviewed by Ginther (1979) indicated that the removal of the foal from the mare at parturition hastened the onset of estrus and caused increased luteinizing hormone levels by the sixth day postpartum.

Hutton and Meacham (1968) examined the effect of mare age on the conception efficiency of various horse breeds. They found that the average conception rates fluctuated between 64% and 79% until the age of eight, then plateaued above 80% from nine until twelve, then gradually declined to 50% for 20 to 24 year old mares. Laing and Leech (1975) found no drop in fertility of Thoroughbred mares until age ten. No significant difference was found among age groups in the incidence of multiple ovulation (Ginther et al., 1982a). Jeffcott and Whitwell (1973) found that the mean age of Thoroughbred mares producing a single conceptus was 11.7 years, whereas the mean age of mares producing twins was 9.6 years. These authors also observed a high incidence of twinning in mares that were four and five years old. Deskur (1985), however, found that the incidence of twinning in Polish Thoroughbred mares was significantly higher for 16 to 20 year old mares than for four to seven year old mares and eight to 11 year old mares. In comparison, the incidence of twinning in cows increases with age (Rutledge, 1975).

Various authors have suggested that increased daylength plays a role in the increase in the incidence of multiple ovulation. Arthur (1958) examined ovaries from slaughterhouse specimens and found that the frequency of twin corpora lutea from one, or a pair of ovaries was most common from June to September. Stabenfeldt et al. (1972) found a lower rate of multiple ovulation in February and March. An examination of breeding records in England by Jeffcott and Whitwell (1973) found that twin ovulations increased from 12% in May to 19% in June. The cases of twins examined in that study were conceived late in the breeding season. Loy (1980) reported that the rate of occurrence of multiple ovulation at the first ovulation postpartum increased from 7.3% in January through March to 21.9% in April and May. A study by Ginther et al. (1982a) found no significant differences between month and rate of occurrence of multiple ovulation, but the role of season in that study was obscured by the use of light treatment on some mares.

The disparity between the multiple ovulation rate and the number of twins aborted or born has led several authors to conclude that one or both embryos in a twin pregnancy undergo embryonic death and are resorbed (Arthur, 1958; Vandeplassche et al., 1970; Stabenfeldt et al., 1972; Merkt and Gunzel, 1979). Evidence presented by Ginther et al. (1982b) suggested that the interval between double ovulations is critical to the establishment of a twin pregnancy. They found that when multiple, synchronous ovulations occur

(those which occur on the same day or one day apart), only one embryo was subsequently found in the pregnant mare. When breeding records were examined for asynchronous, double ovulations (2 to 10 days apart), significantly more twin fetuses were found. Subsequent studies utilizing analyses of breeding records and induced multiple ovulations have supported these findings (Ginther, 1982; Ginther, 1983). If the probability that both ova from a double ovulation had the same chance for fertilization as a single ovum, then double ovulations would be expected to produce a significantly greater number of twin embryos (Ginther et al., 1982b). Pregnancy rates were found to be significantly higher for mares with synchronous, double ovulations (83%) than for mares with a single ovulation (54%). Since no twin pregnancies had resulted from multiple, synchronous ovulations, these authors have suggested that a biological mechanism to eliminate excess embryos exists in the mare. A later study by Ginther (1984b) utilizing ultrasound examination of mares with twin or multiple embryos, indicated that the reduction of the excess embryos occurs after fixation and attachment of the embryos in the uterine horns (16 days). Furthermore, the incidence of embryo reduction was dependent upon whether fixation and attachment was unilateral or bilateral (89% reduction to one embryo versus 11% reduction to one embryo, respectively).

A study by Merkt et al. (1982) was undertaken to

examine the effect of a sudden, temporary reduction of feed intake by mares diagnosed early as carrying twins. The feed reduction was used to increase the chance of resorption of one twin. Serum glucose and fatty acids were measured during the reduced feeding, but no significant differences were found between control and experimental mares. Protein influence was not examined. The authors found that 60% of the mares carrying twins, and given the restricted feed intake, were reduced to a single fetus. The study did not consider the time interval between multiple ovulations, which has been shown to have an apparent effect on reduction of excess embryos (Ginther et al., 1982b).

Stallion sperm cells have been reported to survive up to six days in the mare's reproductive tract (Ginther, 1979) and therefore the possibility exists of sperm surviving long enough to fertilize an ovum from a second, undetected ovulation or from a diestrus ovulation. Veterinarians surveyed by Ginther et al. (1982a) expressed the opinion that twin pregnancies were more likely to occur when an exceptionally fertile stallion was used. Ovulation has been reported to occur in mares with a persistent corpus luteum or in the luteal phase of the estrous cycle (Stabenfeldt et al., 1972; Hughes and Stabenfeldt, 1977). Because several mares were observed to ovulate during the luteal phase of the estrous cycle or when the corpus luteum was regressing, Stabenfeldt et al., (1972) have suggested that mares are less influenced by the suppressive effects

of progestins on ovulation than other domestic species. In most management situations, rectal palpation of the ovaries is discontinued once an ovulation has occurred and a second or diestrus ovulation would not be recorded (Ginther et al., 1982a).

Levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) tend to be reciprocally related during a mare's estrous cycle (Ginther, 1979). Concentrations of FSH follow a bimodal profile during the breeding season (Ginther, 1979). The first increase, which is seasonal, occurs during late estrus and early diestrus. The second FSH surge occurs in mid to late diestrus with a peak occurring 10 to 11 days before the next ovulation. The ovulatory follicle probably originates from the pool of follicles that result from the second FSH surge. Surges of these gonadotropins have been thought to be most closely related with follicular development and multiple ovulation (Urwin and Allen, 1983). Douglas et al. (1974) found that mares had multiple ovulations when given injections of equine pituitary extract. These authors concluded that the ovaries are susceptible to gonadotropin stimulation, but Urwin and Allen (1983) did not find any increase in FSH and LH levels with naturally occurring double ovulations. The authors of the latter study found no correlation between circulating FSH and LH levels during estrous and the number of ovulations, and in fact, most of the twin ovulations observed were associated with lower LH levels.

Twin pregnancies in the mare can be aborted at any stage. Vandeplassche et al. (1970) found that most twin pregnancies resulted in the early resorption of one or both embryos. As previously stated, Ginther et al. (1982b) presented evidence for the natural elimination of one embryo under the proper conditions. Resorption tends to occur more often in lactating mares, a fact supported by the lower incidence of twin pregnancies in lactating mares (Merkt and Gunzel, 1979). For cases in which twin pregnancies do become established and are subsequently aborted, the majority of these abortions occur after eight months, when the greatest period of fetal growth takes place (Whitwell, 1980). Twins that do survive to term are usually smaller and weaker than singletons with birthweights of the two equivalent to the mean weight of singletons (Jeffcott and Whitwell, 1973). Of 124 twin fetuses examined by Jeffcott and Whitwell (1973), only 31 were born alive and of those, 18 survived past two weeks of age.

Placentation

Three types of twin placentation have been described by Jeffcott and Whitwell (1973): type A, in which the twins are dissimilar in size; type B, in which the twins are similar in size; and type C, in which one twin is undergoing mummification and the size difference of the two chorions is greater than in type A. Type A is the most common type encountered. The area of contact between the two chorions is devoid of chorionic villi with type A having the greatest avillous area (Jeffcott and Whitwell,

1973). In 20% of type A placentation, these authors found that it was the smaller twin that was born alive or lived the longest and the larger twin was autolyzed. The reduced chorionic surface area of twin placentae compared to singletons suggests that the twins suffer placental insufficiency and therefore lack adequate nourishment (Franco, 1976; Whitwell, 1980). This is supported by the fact that twins suffer growth retardation and are born small and weak or are stillborn (Mahaffey, 1968; Jeffcott and Whitwell, 1973; Whitwell, 1980). In many cases, one twin will die in utero and become autolyzed or mummified (Mahaffey, 1968; Jeffcott and Whitwell, 1973; Franco, 1976; Whitwell, 1980). The chorion of the surviving twin may expand into the uterine areas previously occupied by the dead twin and may form areas of revascularization with the dead twins chorion which produces the fresh blood that is occasionally found in the chorionic vessels of a mummy fetus (Whitwell, 1980).

Examination of twin chorions reveals areas of fibrous bridging and in some cases, vascular anastomosis (Vandeplassche et al., 1970; Jeffcott and Whitwell, 1973). Jeffcott and Whitwell (1973) reported no widespread anastomosis or appreciable blood chimerism, but Vandeplassche et al. (1970) found blood chimerism in 44% (11 of 25) of the cases examined. Podliachouk et al. found blood chimerism in 22 of 62 cases examined. Although blood chimerism occurs, horse twins usually do not show a freemartin condition and freemartinism has only been reported in two cases

(Vandeplassche et al., 1970). In autopsy examinations of heterosexual twins, female genitalia have been found to be normal both anatomically and histologically (Vandeplassche et al., 1970; Podliachouk et al., 1974). In cattle, anti-Mullerian duct hormone from the male twin causes the regression of Mullerian ducts in both twins, leading to freemartinism in the female twin (Vigier et al., 1984). This event occurs between days 50 and 80 in the gestation period of cattle (Vigier et al., 1984). Vandeplassche et al. (1970) suggested that even though the evidence of blood chimerism can be demonstrated in twin foals, the interchorial anastomosis develops later in the twin foals' fetal life than it does in calf twins.

Plasma cells have been found in the tissue bridges between the chorions, and examination of livers from twin fetuses revealed the presence of a variable number of inflammatory foci (Jeffcott and Whitwell, 1973). No evidence of bacterial or viral infection was found in the placentae, suggesting an adverse immunological reaction between the twins which results in the death of one twin.

Twin case studies

Roberts (1977) described a Thoroughbred twin pregnancy that went 362 days and resulted in a 55 pound, small, thin filly and a mummified male fetus. Addo et al. (1984) reported a case of schistocoelia in a twin foal. These Thoroughbred twins were in the ninth month of gestation with one twin appearing normal and the other smaller and defective. The authors stated that the essential problem

in the defective twin was the lack of fusion of the abdominal wall which eventually lead to the eventration of the abdominal viscera. DiPietro et al. (1983) reported a case of a Standardbred mare which delivered a live 20.4 kg. foal. Nine days later she was examined because of a putrid vaginal discharge and was found to have a retained twin which was abnormally positioned. This 21.8 kg. fetus had to be removed by caesarian section. Based on this case, the authors recommended that whenever a smaller than normal foal is born, the mare should be examined for a second fetus. Asquith and Sharp (1979) reported a case of incomplete twinning resulting in a fetal monster with symmetrical, double development in the cephalic region and a caudal trunk. Double monsters are structurally related to monozygotic twins.

Management of twin pregnancies

When more than one mature follicle is present, management practices to prevent twinning usually involve delay of breeding until the next cycle (Ginther et al., 1982a) or delay of breeding until 12 to 17 hours after ovulation of the first follicle (Pascoe, 1983). Allen (1981) and Belling (1984) have demonstrated that postovulatory breeding can result in conception and Allen (1981) concluded that breeding mares between double ovulations did not necessarily avoid twin conceptions.

Initial diagnosis of twins is usually by rectal palpation which reveals a bilateral twin pregnancy. Diagnosis

of twins in the same horn can be accomplished by use of ultrasound (Pascoe, 1983). If diagnosed early, the pregnancy is usually terminated by administration of prostaglandin F2a, intrauterine infusion with saline, aspiration of the embryo vesicle fluid or the manual crushing of one embryo (Ginther and Douglas, 1982; Roberts, 1982; Pascoe, 1983). However, all of these methods have unsatisfactory results and loss of the breeding season is common. Ginther and Douglas (1982) reported that only four of 11 mares that had twin pregnancies terminated with either prostaglandin or saline infusion, reestablished pregnancy during the same breeding season. The mean day of pregnancy termination in that study was 39 days with a range of 28 to 51 days. These authors also reported that in six of seven mares that had one embryo eliminated by crushing or aspiration, the other embryo was later lost and four of those six mares were not successfully rebred that season. Six of those mares were in days 30 to 38 of gestation and one was at day 49. Roberts (1982) found that blastocyst crushing and rupture of fetal membranes done after day 31 resulted in a rapid fall in survival rate of the uncrushed twin. In some mares, he found that crushing the blastocyst without rupture of the fetal membranes is not possible between days 35 and 45, even with the use of extreme pressure. Sixty percent of the crushed but unruptured blastocysts resulted in resorption of both twins, 20% resorbed without loss of the remaining twin and 20% of the crushed twins survived along with the uncrushed twin. This author feels that the

reasons for losses of the uncrushed twin include pressure transference during the crushing procedure, phagocytosis involved in the resorption of the crushed twin and damage inflicted by endogenous prostaglandin and histamine release. Similar results have been found by Pascoe (1983). He has done work with manual crushing or manipulation of one embryo with administration of meclofenamic acid (a prostaglandin inhibitor) per os and hydroxyprogesterone to support the remaining embryo. One gram of meclofenamic acid was given twice a day for five days prior to crushing followed by one dose per day for four days after the procedure. One gram of hydroxyprogesterone in oil was given the day of the procedure, then repeated every seven days for five treatments. Five of 14 mares treated in this manner retained a single pregnancy, two of the 14 later aborted a single fetus and the remaining seven aborted both twins. Aspiration of the vesicle fluid from one embryo followed by meclofenamic acid and hydroxyprogesterone treatment resulted in the loss of the remaining embryo in five of six mares (Pascoe, 1979). Ginther (1984b) has found that when ultrasound is used to detect early twin pregnancies (12 days), one vesicle can be isolated in one horn and crushed without resorting to the use of antiprostaglandin drugs. Roberts and Myhre (1983) have used supplemental progesterone administration to prevent impending abortion by mares with twin fetuses that are in the latter half of gestation. All three cases reported by these authors went to term and

the mares delivered one viable twin and a mummified fetus.

Parkes and Colles (1977) have shown that fetal electrocardiography can be used later in gestation to detect single or twin pregnancies. Simpson et al. (1982) reported that by using ultrasound, they can diagnose twins as early as 16 days after ovulation and Ginther (1984a) has been able to diagnose twins as early as 12 days.

Abortion

The incidence of abortion in populations of mares has been reported to range from 7% to 17% (Merkt and Gunzel, 1979; Ginther, 1979; Ginther, 1985). Various reasons for abortion, besides twinning, exist including placentitis, viral infections, cord defects and hormonal insufficiencies (Mahaffey, 1968; Platt, 1973; Whitwell, 1980; Ginther, 1985). Reproductive status of the mare was shown to be a significant factor in the incidence of abortion, as well as the interval between parturition and conception (Platt, 1973; Merkt and Gunzel, 1979). Merkt and Gunzel (1979) found that of the Thoroughbred mares that resorbed their pregnancy, the rate of resorption was higher in lactating mares than in nonlactating mares (76.9% versus 23.1%). These authors also found that the incidence of resorption in lactating mares was higher if they were bred on the first or second postpartum estrus. Platt (1973) found that the incidence of abortion in foaling mares conceiving after a postpartum interval of 42 days was lower than in barren mares that had conceived following a season in which they had not conceived or had aborted (6.5% versus 13.6%,

respectively). Pregnancy loss occurred more frequently in older mares, with the incidence being greatly increased in mares older than 18 years (Platt, 1973). The results of a study reviewed by Ginther (1985) indicated that the frequency of abortion was significantly less for mares aged three to six years than for older mares.

Human Chorionic Gonadotropin

Human chorionic gonadotropin (hCG) is administered to mares to hasten ovulation and thereby shorten estrus. Loy and Hughes (1966) treated mares with a single intramuscular injection of 2000 IU of hCG on the second day of estrus which resulted in 88.9% of the treated mares ovulating within 48 hours. Estrus was reduced by 2.4 days in treated mares and no differences in conception rates were observed between treated and control mares. Other workers have produced similar findings. Sullivan et al. (1973) injected mares with 2000 IU of hCG on day two of estrus which resulted in 82.4% of treated mares ovulating by 48 hours with a one day reduction in estrus. Voss et al. (1974) used 3300 IU on day one or two of estrus which resulted in shortening estrus by 3.1 days. Results of studies summarized by Ginther (1979) indicated that doses greater than 4500 IU of hCG were detrimental to conception rates due to the excessive production of estradiol 17B.

hCG is used to increase the ovulation rate in many species, especially when the animal has been primed with pregnant mare serum gonadotropin (Braden et al., 1960;

Killeen and Moore, 1970; Woods and Ginther, 1982; Irvine, 1983). Braden et al. (1960) injected ewes with 1000 IU of hCG and found that the number of ovulations resulting from treatment was greater than in nontreated controls. The authors suggested that in ewes, hCG interacts with the endogenous hormones to cause a greater number of follicles to mature and ovulate. Sasamoto and Taya (1980) found that inducing ovulation with hCG in lactating and immature rats was accompanied by a significant surge in follicle stimulating hormone. Killeen and Moore (1970) found that hCG administration increased the proportion of follicles which mature and ovulate and increased the incidence of multiple births in ewes. Woods and Ginther (1983) found that hCG administration to mares treated with equine pituitary extract shortened the interval between the first and last ovulation. Irvine (1983) cited unpublished data that indicated that hCG administration to Thoroughbred mares, however, does not appear to be associated with twinning.

## MATERIALS AND METHODS

Breeding records for the years 1980 through 1984 were obtained from a Michigan Standardbred breeding farm. Information was collected in the following categories: 1) mare reproductive status and age, 2) the number of ovulatory periods for each mare, 3) the number of follicles per ovulatory period, 4) the number of ovulations per ovulatory period, 5) administration of hCG, and the amount given, 6) the pregnancy status for each mare, and whether or not she subsequently aborted.

Reproductive status in this study was defined as one of three possibilities: 1) foaling mares were those that had foaled during the current breeding season, 2) barren mares were those that had been bred the previous season and had not conceived or had lost their pregnancy prior to the current breeding season, 3) maiden mares were those mares that had not been previously bred. On the basis of studies by Hutton and Meacham (1968) and Laing and Leech (1975), the mares were classified into one of four age groups: 1) 2 to 7 years, 2) 8 to 12 years, 3) 13 to 19 years and 4) 20 years and older.

An ovulatory period was defined on the basis of the number of ovulations detected by rectal palpation during the period when estrus was positive. Each ovulatory period

was treated independently, even though a given mare may have contributed more than one period and any ovulatory period with an unknown number of ovulations was excluded from the analysis. Some mares experienced prolonged estrus of up to several weeks during which time the mare may have been bred every other day or at sporadic intervals. The number of follicles and the number of ovulations per ovulatory period were based upon rectal palpation. Single ovulations were recorded if evidence of an ovulation had been noted. If one follicle was present, but was not recorded as ovulated, a single ovulation was assumed if the mare was subsequently diagnosed as having a single pregnancy. Accepted double ovulations were determined by the palpation record of two ovulations whereas suspected double ovulations were determined by the disappearance of one large follicle (>30mm.) plus a recorded ovulation, or the disappearance of two large follicles. Actual palpations were not performed daily or even every other day in most cases, with gaps of up to five days not being unusual. During the years of the study, three different veterinarians were employed by the farm. From 1980 to mid 1982, one veterinarian performed all of the palpations. Rectal palpations were not done as frequently as in subsequent years and consequently, records were incomplete and some follicular information was not verified. In 1982, two other veterinarians were involved in the palpations and ovaries were checked more often. The same veterinarian performed the palpations in 1983 and 1984 and the records were more

complete. Mares with double ovulations were usually bred unless the management felt that the situation might present a twin pregnancy, and in that case, they would discontinue breeding after one ovulation had occurred.

Mares were exposed to a stallion (teasing) on an every other day basis with inseminations done on the days the mares were not teased. In the majority of the cases, mares were inseminated every other day until they went out of estrus. Mares that were bred, diagnosed as pregnant, aborted, then were bred again during the same season, were counted twice.

Dosage of hCG was recorded to determine if it had an effect on the development of multiple follicles and multiple ovulations. Single dosage levels were 2500, 3000, 3300, 5000, 10,000 IU, or the amount was unknown. Only a few mares received multiple doses during a given ovulatory period and these were grouped with mares that received a single dose for analysis.

The pregnancy status for mares was coded for single and twin pregnancies, as well as mares that failed to conceive. There were instances when the pregnancy status of mares was unknown because those mares had left the farm before a determination of pregnancy status was made. Later reports by owners to the farm were entered into the records but the pregnancy status for a number of mares would remain unknown until those mares had foaled, or returned for rebreeding during the following breeding season. The

majority of unknown outcomes were from the 1984 breeding season. Prior to 1984, pregnancy was determined at the farm by rectal palpation. In 1984, in some cases, ultrasound examinations supplemented rectal palpation for pregnancy determination. In general, twin pregnancies were not interrupted by management practices but in the 1984 breeding season, a diagnosed twin pregnancy was terminated by the administration of prostaglandin.

Abortions were either observed by the farm staff or were later reported to the farm by individual mare owners. Although the point in gestation that the abortions occurred was known for a portion of the mares, for others this was unknown and the time of occurrence of the abortions was not analyzed.

A microcomputer program, written in the turbo pascal language, was developed to ask the user a series of questions concerning the breeding records that were examined. Records on each mare were written onto a data file for storage and a number was assigned to each mare's record. A second program was developed to output the data onto a hard copy in order to edit each record for typographical or input errors. A third program was written to correct errors and complete the deletions in the record file. Once all the corrections had been made, several programs were developed to compile the data. Those results were then printed onto a hard copy, totals were calculated and statistical analyses were performed.

Unknown outcomes of breedings were excluded in

determining conception, twinning and abortion rates. Pregnancy rates were calculated by dividing the number of ovulatory periods in which a conception occurred by the total number of ovulatory periods. Conception rates were calculated by dividing the number of mares in foal by the number of mares bred. Twinning rates were calculated by dividing the number of twin pregnancies by the number of mares in foal. The abortion rates were calculated in a similar manner. Chi-square tests using contingency tables of r by c (where r is equal to the number of rows and c is equal to the number of columns) were used to determine if reproductive status or age groups were independent of follicle number, ovulation rate and abortion rate. Twenty mares with synchronous, double ovulations and one mare with an asynchronous, double ovulation with a two day interovulatory period had been treated with hCG prior to ovulation with the dosages ranging from 2500 IU to 5000 IU. A contingency table was used to determine if ovulation rate was independent of hCG dosage. When significant differences were found, the Bonferroni method of testing nonindependent contingency tables was used.

## RESULTS AND DISCUSSION

Data obtained from breeding records is subject to confounding effects. The records for this study were obtained from one farm in an attempt to reduce confounding due to differences between farms. There was more than one veterinarian involved in performing the palpations and there were differences in management practices. Detection of ovulation and pregnancy by rectal palpation is subject to error. The effect of month on multiple ovulation was not examined because lights were used on some of the mares to induce the early onset of the ovulatory season.

The incidence of multiple ovulation in the Michigan population of mares was 7.6% with no triple ovulations observed (Table 1). Suspected double ovulations accounted for nearly half of the total and were included in the analyses.

Pregnancy rates for mares with synchronous, double ovulations and double ovulations with a two to three day interval between them were higher than for mares with a single ovulation (Table 2). This trend of a higher pregnancy rate for mares with synchronous, double ovulations is similar to that reported by Ginther et al. (1982a), who found an 83% pregnancy rate for mares with synchronous, double ovulations compared to 54% pregnancy rate for mares

Table 1: Frequency of single and double ovulations

| No. ovulations/ovulatory period | | |
|---|---|---|
| one | 1685 | (92.4%) |
| two | 139 | (7.6%) |
| accepted | 76 | (54.7%) |
| suspected | 63 | (45.3%) |

Table 2: Relationship between the number of ovulations and number of embryos

| No. of ovulations | No. of embryos one | two | Pregnancy rate |
|---|---|---|---|
| one | 681 | 3 | 42.2% |
| two (a,b) | | | |
| 0-1 day interval | 30 | 1 | 63.3% |
| 2-3 day interval | 9 | 0 | 64.3% |
| 4-5 day interval | 8 | 0 | 47.0% |
| 6-7 day interval | 10 | 0 | 43.5% |
| 8-10 day interval | 6 | 1 | 70.0% |
| unknown | 309 | 4 | 30.4% |

a. includes suspected double ovulations
b. double ovulations were excluded if the mare was not bred or breeding was delayed until after one ovulation.

with single ovulations. The 70% pregnancy rate for mares with double ovulations that were eight to ten days apart was unexpected and may have been due to chance (Table 2).

The incidence of single or multiple follicles was found to be dependent upon reproductive status (Table 3). Results of specific comparisons indicated differences between foaling and barren or maiden mares. Previous workers have found a significantly lower incidence of multiple ovulation in foaling mares than in barren and maiden mares (Ginther et al., 1982a; Ginther, 1983). A suppressive effect of nursing on ovulation may be involved (Ginther, 1983). The incidence of multiple ovulation was lower for foaling mares (6.6%) than for barren mares (9.9%) in this study, but reproductive status was found to be independent of the ovulation rate (Table 4), arguing against a suppressive effect of nursing. The incidence of single and multiple follicles was found to be independent of age group (Table 5) as was the ovulation rate (Table 6). Ginther et al. (1982a) also found no effect of age group on ovulation rate.

The 0.8% incidence of twins found in this population (Table 7) falls within the estimates of 0.4% and 1.5% found in a study of Standardbred mares reported by Ginther et al. (1982a). Estimates of twinning, based upon externally observed twin abortions or births, were 0.2% and 0.3% for Arabian mares, 0.5% to 1.0% for Quarter Horse mares, and from 0.2% to 4.5% for Thoroughbred mares (Ginther et al., 1982a). Jeffcott and Whitwell reported that no sets of

Table 3: Effect of reproductive status on the incidence of single and multiple follicles

| Reproductive Status | No. ovulatory periods with Single follicle | Multiple follicles |
|---|---|---|
| Foaling | 727 (75.7%) | 234 (24.3%) |
| Maiden | 183 (60.6%) | 119 (39.4%) |
| Barren | 453 (56.2%) | 353 (43.8%) |

Chi-square statistic = 78.14, C.V. = 13.82 (P<.001)
Specific comparisons between foaling and maiden and foaling and barren mares were found to be significant (P<.01), but no differences were found between barren and maiden mares.

Table 4: Effect of reproductive status on ovulation rate

| Reproductive Status | No. ovulatory periods with one ovulation | two (a) ovulations |
|---|---|---|
| Foaling | 819 (93.4%) | 58 (6.6%) |
| Maiden | 229 (92.7%) | 18 (7.3%) |
| Barren | 637 (91.0%) | 63 (9.0%) |

Chi-square statistic = 3.199, C.V. = 5.991 (not significant at P<.05).
a. includes suspected double ovulations

Table 5: Effect of age group on the incidence of single and multiple follicles

| Age group | No. ovulatory periods with Single follicle | Multiple follicles |
|---|---|---|
| 2-7 years | 394 (66.0%) | 203 (34.0%) |
| 8-12 years | 444 (65.3%) | 236 (34.7%) |
| 13-19 years | 423 (64.7%) | 231 (35.3%) |
| 20+ years | 101 (73.7%) | 36 (26.3%) |

Chi-square statistic = 4.294, C.V. = 7.815 (not significant at $P<.05$).

Table 6: Effect of age group on ovulation rate

| Age group | No. ovulatory periods with one ovulation | two (a) ovulations |
|---|---|---|
| 2-7 years | 475 (92.8%) | 37 (7.2%) |
| 8-12 years | 549 (90.9%) | 55 (9.1%) |
| 13-19 years | 547 (93.3%) | 39 (6.7%) |
| 20+ years | 114 (93.4%) | 8 (6.6%) |

Chi-square statistic = 3.001, C.V. = 7.815 (not significant at $P<.05$).

a. includes suspected double ovulations

twins had been found in a herd of crossbred pony mares during a 19 year period. There is a high degree of repeatability of double ovulations and twinning within mares and within family lines (Ginther, 1982), which would account for differences seen within the same breed.

No conclusions can be drawn about the effect of time interval between double ovulations on the number of embryos because most of the twin pregnancies occurred as the result of a recorded single ovulation, or an unknown number of ovulations (Table 2). Once an ovulation was recorded, palpation was discontinued unless the mare had another large follicle present, or had a history of prolonged estrus. Therefore, lack of detection of a second or diestrus ovulation would account for twins resulting from a reported single ovulation. Other workers have found that twins were more frequently diagnosed when the double ovulations were asynchronous (Ginther et al., 1982b; Ginther, 1983). These investigators also found no twin pregnancies from mares with synchronous, double ovulations. In the present study, however, one such twin pregnancy was found (Table 2). Testing for significance was not done due to the small number of twins found as a result of double ovulations. The embryo reduction mechanism described by Ginther et al. (1982b) and Ginther (1983) apparently is more effective when the embryos are the same age or a day apart. Embryo reduction occurs more frequently if the twin vesicles are unilaterally positioned in the mare's uterus

than if they are positioned bilaterally (Ginther, 1984b). Ginther (1984b) concluded that when twins are not corrected by the embryo reduction mechanism by day 40 of gestation and enter the fetal stage, they are more likely to be aborted than undergo reduction or birth of twins. The twins in this study were diagnosed by ultrasound at 60 days, but no record of their position in the uterus was made. The mare was given prostaglandin to abort the pregnancy and was successfully rebred the same season.

Ginther (1983) reported a lower incidence of twinning in foaling mares than in barren or maiden mares, but both foaling and barren mares had a 0.9% incidence of twinning in this study (Table 8). The incidence of twinning tended to decrease with age group except for the oldest group of mares (Table 9). The 2% incidence of twins found in these mares could be due to only one observation in the group. Although only a few twin pregnancies were found in this population, the higher incidence of twins in the younger mares confirms a report by Jeffcott and Whitwell (1973) who found that the mean age of mares producing a single conceptus was higher than that of mares producing twins.

The overall conception rate for this population of Standardbred mares was 75.6%. There were 82 mares with unknown outcomes of breeding, but even if all of those mares were in foal, the conception rate would only be increased to 77.4%. The abortion rate for the mares bred at the farm was 12.9% over the five years studied.

The conception rates for mares classified by

Table 7: Incidence of single and twin pregnancies

| | No. mares |
|---|---|
| single | 1053 (99.2%) |
| twin | 9 (0.8%) |

Table 8: Incidence of twins according to reproductive status

| Status | No. twin pregnancies/ No. mares in foal |
|---|---|
| Foaling | 5/577 (0.9%) |
| Maiden | 1/136 (0.7%) |
| Barren | 3/349 (0.9%) |

Table 9: Incidence of twins according to age group

| Age group | No. twin pregnancies/ No. mares in foal |
|---|---|
| 2-7 years | 3/294 (1.0%) |
| 8-12 years | 3/372 (0.8%) |
| 13-19 years | 2/343 (0.6%) |
| 20+ years | 1/52 (2.0%) |

reproductive status ranged from 70.5% for maiden mares to 78.3% for barren mares (Table 10). Ginther (1979) reported that, in general, the conception rate for barren mares was lower than for lactating mares. Hutton and Meacham (1968) reported similar findings. Abortions were more common in mares that conceived at the first or second postpartum estrus (Platt, 1973; Merkt and Gunzel, 1979). In the present study, the effect of conceptions at the first postpartum estrus on the incidence of abortion was not examined. No effect of reproductive status on abortion was found (Table 10). In a previous study, abortion rate was lower for lactating mares than for barren mares (Hutton and Meacham, 1968).

Conception rates peaked at 78.3% for the eight to 12 year old group of mares, then declined to 66.7% for the oldest group of mares (Table 11). The decline is similar to, but not as dramatic as that noted by Hutton and Meacham (1968). In that study, eight to 12 year old Thoroughbred mares had a conception rate that ranged from 80.4% to 89.6%, with conception rates falling to 50% for mares that were 20 years and older. Twenty-two year old mares from the Michigan farm had an unusually high conception rate (84.2%) for that age and this contributed to the higher conception rate observed in the older mares.

The incidence of abortions increased as the age group of the mares increased, with the 20 year old and older group experiencing a 23.1% pregnancy loss (Table 11).

Table 10: Conception rate and incidence of abortion according to reproductive status (a)

| | Status | | |
|---|---|---|---|
| | Foaling | Maiden | Barren |
| No. mares in foal | 577 | 136 | 349 |
| % conception | 76.2% | 70.5% | 78.3% |
| No. of abortions | 73 | 13 | 51 |
| % abortions (b) | 12.7% | 9.6% | 14.6% |

a. includes mares that were bred, aborted and rebred.
b. Chi-square statistic = 2.276, C.V. = 5.991 (not significant at P<.05).

Table 11: Conception rate and incidence of abortion according to age group (a)

| | Age group | | | |
|---|---|---|---|---|
| | 2-7 | 8-12 | 13-19 | 20+ |
| No. mares in foal | 294 | 372 | 343 | 52 |
| % conception | 74.2% | 78.3% | 76.9% | 66.7% |
| No. of abortions | 32 | 45 | 48 | 12 |
| % abortions (b) | 10.9% | 12.1% | 14.0% | 23.1% |

a. includes mares that were bred, aborted and rebred.
b. Chi-square statistic = 7.972, C.V. = 7.815 (P<.05) Specific comparisons between the youngest and the oldest mares approached a significant level (P<.05). No differences were found between the other age groups and the oldest group.

Although a significant effect of age group on abortion rate was found, when each of the three younger age groups, and all three younger age groups combined, were compared to the 20 year old and older age group, only the comparison of the youngest and the oldest age group approached a significant level ($P<.05$). The pattern of an increasing abortion rate with aging is consistent with the results of previous workers who found a higher incidence of abortion in older mares (Platt, 1973; Ginther, 1985).

The effect of hCG on follicular development, ovulation and multiple births has been demonstrated in other species, particularly the ewe (Braden et al., 1960; Killeen and Moore, 1970). No data were found in the literature that would indicate that such an effect exists in mares. In this study, ovulation rate was found to be independent of hCG dosage (Table 12).

Table 12: Effect of hCG dosage on ovulation rate

| | No. ovulatory periods with one ovulation | two ovulations |
|---|---|---|
| hCG | 244 (92.1%) | 21 (7.9%) |
| no hCG | 1441 (93.9%) | 94 (6.1%) |

Chi-square statistic = 1.246, C.V. = 3.841
(not significant at $P<.05$).
Dosage levels of hCG ranged from 2500 IU to 10,000 IU.
Double ovulations with one ovulation stimulated by hCG were excluded.

## SUMMARY

The multiple ovulation rate for this population of Standardbred mares was 7.6%. An effect of reproductive status on the incidence of single and multiple follicles was found with significant differences between foaling and barren or maiden mares. However, ovulation rate was independent of reproductive status, even though foaling mares had a lower percentage of double ovulations than barren or maiden mares. Ovulation rate was independent of hCG dosage.

Pregnancy rates tended to be higher for mares with synchronous, double ovulations than for mares with single ovulations. No conclusions can be made about the effect of the time interval between double ovulations and the number of embryos. No differences were seen between foaling and barren mares in the incidence of twinning. If the oldest group of mares is not considered, then the youngest age group of mares had the highest incidence of twinning for the population.

Conception rates were the lowest for maiden mares and the highest for barren mares. Foaling mares had a conception rate that was slightly lower than that for barren mares. Conception rates were highest for the eight to 12 year old mares and lowest for the mares that were twenty

years old and older. Reproductive status was independent of the incidence of abortion, but age group was not. However, only the comparison of the youngest and the oldest mares approached significance ($P<.05$).

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