Reduced Reproductive Efficiency in the Aged Mare: Role of Early Embryonic Loss  (30 Mar 2000)

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Introduction

In contrast to other domestic livestock, the horse is maintained as part of the breeding population for a relatively longer period of time. In many situations, older mares may be more valuable because of their proven produce record, and it is common to find mares that are bred at greater than 20 years of age. For these reasons, age-related reproductive failure has received considerable study. This paper will summarize our current understanding of reproductive failure in aged mares, particularly as it relates to embryonic loss.

Foaling rates decline with maternal age after 14 to 16 years [1, 2]. Although pregnancy losses during late gestation increase in older mares, it appears that failures of early pregnancy account for the majority of the reduced fertility in aged mares. The widespread application of transrectal ultrasonography for pregnancy detection in mares at Days 10 to 14 after ovulation led to evaluation of the incidence of embryonic loss in mares between Days 14 and 40. These studies indicate that overall pregnancy rates declined and that detected embryonic loss rates increased with increasing mare age [2]. Data from field studies such as these provided a strong impetus to examine very early embryonic mortality in mares.

Incidence of Early Embryonic Loss

Prior to Day 10, ultrasonographic evaluation cannot detect the early equine conceptus; however, several reports based upon embryo collection and transfer provide information regarding very early embryonic loss in mares. From these studies, it appears that there is a sharp decline in the recovery rate of blastocysts from the uterus of aged mares [3, 4]. In addition, blastocysts from aged mares have more morphologic defects [4], and fewer blastocysts from aged mares survive after embryo transfer to healthy recipient mares [3]. Because the equine embryo enters the uterus at Day 5.5, it was unclear from these observations whether the decline in number and apparent viability of blastocysts from aged mares was related to an abnormal uterine environment, abnormalities of the uterine tubal environment or abnormalities inherent in the embryo.

Several studies have characterized the early equine embryo during uterine tubal passage in mares of different ages and fertility status. As in other species, the overall fertilization rate in young mares appears high (> 90%) based upon the cleavage rate of ova recovered two days after fertilization whereas the fertilization rate in aged mares was 80 to 90%. Estimates of the embryonic loss rate between fertilization and Day 10 were 9% for young mares compared to 60 to 70% for aged mares [5, 6, 7]. Although pregnancy rates at Day 2 were similar in young and aged mares, by four days after fertilization, there was a significant reduction in pregnancy rates in aged mares. This finding suggested that the interval between Day 2 and 4 might represent a critical period in pregnancy failure in aged mares. These studies confirmed the importance of very early embryonic loss in the reduced fertility of aged mares; however, such studies do not provide information as to the causation of such losses.
Factors in Embryonic Loss

Clinically, abnormalities of the endometrium have been considered an important factor in the reduced fertility of aged mares. A number of studies have characterized degenerative changes in the equine endometrium as a function of increased mare age, and the decline in mare fertility with increased age has been frequently attributed to these changes. These changes include endometrosis, endometritis as well as vascular changes. We examined the impact of uterine environment on embryo survival in young and aged mares in which endometrial histopathology was evaluated [8]. As expected, aged mares had a higher incidence of degenerative and inflammatory changes in the endometrium. When morphologically normal, Day-7 or -8 blastocysts were transferred into the uterus of young and aged recipient mares, embryo survival rates at Days 12 and 28 were not different between young and aged mares (55% vs 45% at Day 12). This study indicated that uterine effects did not play a major role in the high incidence of early embryonic loss that had been previously detected in aged mares. The impact of uterine abnormalities may be more likely during later periods in gestation in these mares.

The role of pre-uterine events in embryonic wastage in aged mares was examined by collection of embryos from the uterine tube of aged and young mares at four days after fertilization [6]. The viability of these embryos was assessed after transfer to the uterus of young recipient mares. The survival of embryos from aged mares was significantly lower than those from young mares after placement into a normal uterine environment, which indicates a role for either the uterine tubal environment or embryonic defects for early losses in aged mares.

We hypothesized that initiation of embryonic transcription during early embryogenesis could be one factor associated with embryonic mortality in aged mares, particularly in view of the apparent reduction in embryo viability as soon as four days after fertilization. We examined the initiation of transcription in early cleavage stage embryos to determine the timing of activation of the embryonic genome. Based upon incorporation of $^3$H-uridine, the initiation of transcription occurs between the 4 - 8-cell stages [9]. We then examined the in vitro development of early embryos from both aged and young mares during coculture with uterine tubal epithelial cells. There was no catastrophic failure of development of embryos from aged mares during in vitro culture as the developmental rate of embryos from young and aged mares to blastocysts was similar. However, embryos from aged mares had fewer blastomeres and poorer quality scores than did embryos from young mares [7]. This study supported a reduction in embryo quality in embryos from aged mares during the preblastocyst period, but failed to identify a block to development of early cleavage-stage embryos corresponding to the initiation of embryonic transcription.

Two studies were conducted to evaluate the role of the uterine tubal environment on early embryonic loss in aged mares. In the first study, histopathologic evaluation of both the ampulla and isthmic regions of the uterine tube was conducted on aged and young mares. In contrast to the findings associated with the endometrium, there was no association between mare age and the incidence of salpingitis. In a second study, differences in proteins synthesized and secreted in vitro by explanted oviductal tissue was examined by incorporation of $^{35}$S-methionine, two-dimensional polyacrylamide gel electrophoresis and fluorography. Both qualitative and quantitative differences in the patterns of proteins from uterine tubal epithelium were detected between young and aged mares; however, the importance of these differences relative to embryonic development remains to be determined [10].

Role of Oocyte Abnormalities

Deterioration of oocytes associated with increased maternal age has been well described in other species. Unfortunately, few studies have addressed this issue in the horse. In one study, we examined the in vitro maturation of oocytes from young and aged mares as well as the rate of aneuploidy in oocytes from each group. Oocytes from aged mares had a delayed maturation to metaphase II compared to oocytes from young mares Unfortunately, inadequate numbers of chromosomal spreads were available to assess the rate of aneuploidy in oocytes from these two groups of mares, and the relative incidence of aneuploidy in oocytes from aged mares remains to be defined [11]. In another report, Carnevale and Ginther reported the results of gamete intrafallopian transfer studies in which oocytes were collected from both young and aged mares and transferred into the uterine tube of young recipient mares [12]. Oocytes from aged mares resulted in
significantly fewer pregnancies than those from young mares after transfer to the uterine tube of a young recipient mare. This study provides the most convincing evidence to date of an age-related decline in oocyte quality in mares as a major factor in the reduced fertility of these mares. It appears likely that this reduction is secondary to an increased incidence of aneuploidy in oocytes from aged mares; however, this remains to be established for the horse. Delayed ovulation and preovulatory aging of oocytes has been proposed as one factor associated with abnormal oocytes in aged females. Aged mares appear to undergo a reproductive senescence characterized by lengthening of the follicular phase, irregular ovulations and eventually follicular inactivity [13,14]. The onset of these changes appears to occur over a relatively broad age range but appears most commonly in mares > 20 years of age. Prolongation of the follicular phase appears to be associated with an elevation of both FSH and LH in aged mares [14]. The relationship of declining follicular populations, altered gonadotropin levels, and prolonged follicular development in aged mares with an increased incidence of abnormal oocytes remains to be explored as an explanation for age-related infertility in mares.

Conclusions
In summary, there is a well-characterized decline in fertility in mares associated with increased maternal age. Much of the reduced fertility in aged mares can be attributed to early (preblastocyst) embryonic wastage. From currently available data, it appears that oocyte defects are the most likely cause of the increased reproductive wastage in aged mares, although alterations in the uterine tubal environment have been demonstrated in aged mares. It appears likely that the abnormal oocytes in aged mares are related to an increased incidence of aneuploidy; however, this remains to be confirmed experimentally. Alterations in follicular growth in aged mares might be associated with the increased incidence of abnormal oocytes and testing this hypothesis should be a high priority in the study of aged-related infertility in mares.

References


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