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Incidence and morphology of anovulatory haemorrhagic follicles in the mare

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Summary

Retrospective data from 47 experimental mares monitored during a total of 737 oestrous cycles over 3 years were analysed. During the breeding season (April-August), development of an anovulatory haemorrhagic follicle (AHF), with a mean diameter of 48.1 ± 8.0 mm, occurred in 11.9 % of the oestrous cycles. In most cases, the dominant follicle grew normally to a diameter Δ 45 mm (range 38-65 mm), oedema developed in the endometrium as expected but ovulation did not occur. Instead, the follicular fluid became hazy in appearance and increasingly filled with echogenic flecks and strands. The concentration of progesterone in the mare's serum; i) rose slowly and progressively (50.8 %); ii) remained < 1 ng/ml (11.1 %); iii) plateaued between 1 and 4 ng/ml (25.4 %); or iv) rose suddenly from 0 to > 10 ng/ml in < 24 hrs (12.7 %). Most AHFs that were of AHF formation from one breeding season to the next. Aged mares tended to develop unusually large (> 50 mm) and hazy AHFs, whereas younger maiden fillies tended to produce medium-sized AHFs that became more echogenically dense. During the transitional phase in Autumn, AHF formation occurred in 22.2 % of oestrous cycles in September and October but the occurrence peaked at 58.8 % in November.

Keywords: ovulation failure, anovulatory haemorraghic follicle

Vorkommen und Morphologie von anovulatorischen hämorrhagischen Follikeln bei Stuten

Retrospektiv wurden die Daten von 47 Stuten aus Versuchsreihen, mit insgesamt 737 Zyklen über den Zeitraum von 3 Jahren, ausgewertet. Während der Zuchtsaison von April bis August entwickelte sich in 11,9 % der Zyklen ein anovulatorischer hämorragischer Follikel (AHF) mit einem Durchmesser von 48,1 ± 8,0 mm. In den meisten Fällen wuchs der dominante Follikel auf einen Durchmesser Δ 45 mm (38-65 mm). Obwohl erwartungsgemäß eine Ödematisierung des Uterus auftrat, fand eine Ovulation nicht statt. Stattdessen erschien die Follikelflüssigkeit im Ultraschall verschwommen mit einer Zunahme echoreicher Anteile. Die Serumprogesteronkonzentration zeigte: 1.) in 50,8 % der Fälle einen langsamen, stetigen Anstieg; 2.) in 11,1 % ein Verbleiben der Konzentration auf < 1 ng/ml; 3.) in 25,4 % ein Plateau zwischen 1 und 4 ng/ml oder 4.) in 12,7 % einen plötzlichen Anstieg der Progesteron Konzentration von 0-10 ng/ml innerhalb eines Tages. Die größte Anzahl der AHF's, die mit hohen Plasmaprogesteronkonzentrationen einhergingen, wiesen im Ultraschall eine starke Echogenität auf. Nach i.m. Injektion eines PGF Analogon zeigten diese Stuten einen Östrus mit folgender normaler Ovulation. Insgesamt 50,5 % der Stuten entwickelten einen AHF während mindestens einem, 26,2 % während mindestens zwei Östruszyklen der Zuchtsaison. Bei einzelnen Stuten konnte eine deutliche Tendenz für das Wiederauftreten von AHF´s in der folgenden Zuchtsaison festgestellt werden. Ältere Stuten neigten zur Ausbildung von ungewöhnlich großen (> 50 mm) und verschwommenen Follikeln, während junge Maidenstuten gewöhnlich mittelgroße AHF´s mit einer verstärkten echogenen Dichte entwickeln. Während der Übergangsphase im Herbst kam es im September und Oktober bei 22,2 % der Zyklen zur AHF Bildung. Ein Peak von 58,8 % AHF Bildung zeigte sich im November.

Schlüsselwörter: Reproduktion, Ovulation, ausbleibende Ovulation, anovulatorischer hämorrhagischer Follikel

Introduction

During the dioestrous phase of the mare's oestrous cycle, a cohort of 8-12 ovarian follicles is stimulated to develop by the action of pituitary FSH. During the ensuing oestrus, a "dominant follicle" is selected to continue maturation towards ovulation under the influence of pituitary LH (*Evans* and *Irvine* 1975), while the other follicles in the cohort become atretic. Morphological and biochemical changes in the follicular wall result in follicular rupture (ovulation) through the ovulation fossa and the release of the oocyte surrounded by cumulus cells into the oviduct. Ovulation failure has been well documented in the mare (*Ginther* 1979, *Bosu* et al. 1982, *Ginther* and *Pierson* 1989, *Daels* and *Hughes* 1993, *Pierson* 1993, *McCue* and *Squires* 2002), the most common form of which is the development of an anovulatory haemorrhagic follicle (AHF) where the follicle fails to rupture and its cavity becomes increasingly filled with blood. Such haemorrhagic follicles are commonly observed during the transitional phases in Spring and Autumn (*Ginther* 1992, *Nunes* et al. 2002), when the secretion rates of both gonadotropins and ovarian steroids are declining (*Daels* and *Hughes* 1993). Their occurrence during the breeding season greatly reduces breeding efficiency and the aim of the present study was to document the incidence of AHFs in an experimental herd of mares undergoing repeated pharmacological shortening of their oestrous cycles.

Materials and methods

Data from 47 experimental Thoroughbred mares monitored during a total of 737 oestrous cycles over 3 consecutive breeding seasons were analysed retrospectively. Oestrous mares were scanned every other day until the dominant follicle reached a diameter of > 30 mm, when they were scanned every day until ovulation and a jugular vein blood sample was collected daily until 3 days after ovulation. The samples were centrifuged and the serum decanted and stored at -20°C until assayed for progesterone concentration, using the Amplified Enzyme-Linked Immunoassay (AELIA) described by *Allen* and *Sanderson* (1987). Follicular diameter and texture, as well as the degree of endometrial oedema were recorded, together with any hormone treatments administered either to induce luteolysis and bring the mare back into oestrus (prostaglandin F analogue, cloprostenol; Estrumate: Coopers Animal Health Ltd., Cheshire, UK) or to hasten ovulation (human Chorionic Gonadotrophin, hCG; Chorulon, Intervet UK Ltd., Buckinghamshire, UK; or Crude Equine Pituitary Gonadotrophin; CEG, INRA, Nouzilly, France).

Results

Throughout the breeding season (April-August), an anovulatory haemorrhagic follicle (AHF) developed in 11.9 % of the

oestrous cycles. In most cases, the dominant follicle grew normally to a mean diameter of 48.1 ± 8.0 mm (range 38-65 mm), oedema developed in the endometrium as expected but ovulation did not occur. Instead, free-floating echogenic flecks and strands began to appear within the anechogenic follicular fluid, the amount of which increased with further follicular growth. In most cases, the echogenic flecks were first observed in the follicular fluid at the end of oestrus when the follicle had already reached a diameter Δ 35 mm and the blood progesterone concentration was still < 1 ng/ml. Sometimes however, the same chain of events was seen in smaller follicles (25 mm diameter) when the density of flecks increased as the follicle grew, in parallel with a progressive increase in blood progesterone concentrations.

Most AHFs (88.1 %) were accompanied by an elevated serum progesterone concentration and they responded well to an intramuscular injection (i.m.) of 250 µg of the prostaglandin F analogue, cloprostenol (Estrumate: Coopers Animal Health Ltd., Cheshire, UK) by returning to oestrus and ovulating normally. The blood progesterone level either rose very steadily to reach values > 8 ng/ml (50.8%), or the concentration plateaued between 1 and 4 ng/ml (25.4 %). 7/63 (11.1 %) of the AHFs did not form luteal tissue and the serum progesterone concentration remained < 1ng/ml. But in 8/63 (12.7 %) of the cases, the AHF was formed much more rapidly with the appearance of a dense coalescence of echogenic flecks in the follicular fluid in < 24 hrs. These structures were somewhat similar in general appearance to a corpus haemorrhagicum but could be differentiated by the lack of any change in appearance during the following days and a sudden very rapid rise in blood progesterone concentrations from < 1 to > 10 ng/ml in < 24 hrs.

A total of 50.5 % of the mares exhibited an AHF during at least one oestrous cycle, and around 26.2 % produced an AHF during at least 2 oestrous cycles. A definite tendency for recurrence of AHF formation from one breeding season to the next was noted in individual mares.

Young maiden fillies (3-5 years old) tended to produce medium-sized AHFs that became progressively more echogenically dense while aged mares (> 15 years) tended to develop unusually large (> 50 mm) follicles containing hazy follicular fluid due to a lower density of small echogenic flecks. The preovulatory follicle in these cases never showed the characteristic wedge shape created by pointing of the follicle towards the ovulation fossa, although the follicular wall became thickened and the endometrium became oedematous. Such AHFs usually persisted for several weeks, producing low levels of progesterone in the serum (1-3 ng/ml) over the first 5-7 days but then rising to higher values (7-8 ng/ml) which then persisted for the remainder of their lifetime.

During the transitional phase in Autumn, AHF formation occurred in 22.2 % of oestrous cycles in the months of September and October, reaching a peak occurrence as high as 58.8 % in November.

Discussion

While failure of ovulation occurs commonly during the transitional spring and autumn periods, its occurrence during the natural breeding season seriously jeopardises reproductive efficiency. In the present study, the incidence and appearance of AHFs was documented in experimental mares that were being treated repeatedly with a prostaglandin F analogue to induce luteolysis and thereby shorten dioestrus and with a variety of gonadotropins preparations to hasten ovulation. Overall, an AHF developed in 11.9 % of all the oestrous cycles monitored, which is higher than the 8.2 % incidence recorded by *McCue* and *Squires* (2002) and 4.7 % recorded by *Ginther* and *Pierson* (1989). The higher incidence in our mares may well have been related to the repeated use of luteolytic and ovulation inducing drugs in an experimental situation designed to short cycle the mares and synchronise their ovulations.

Ultrasonographic prediction of ovulation failure remains extremely difficult since there is no precursor signs of AHF formation and most of the normal preovulatory characteristics are usually present. As many as 88.1 % of the AHFs that formed were accompanied by the development of luteal tissue which then made the AHF responsive to the administration of a prostaglandin F analogue. However, there still remained the high probability that the same individual would develop further AHFs during the same breeding season.

Most preovulatory follicles that failed to ovulate and filled with blood in this manner had not responded to the administration of hCG or CEG. Hence it remains impossible to predict formation of AHFs. However, provided the AHF secreted reasonable quantities of progesterone it proved possible to use the mare as a recipient in an embryo transfer programme. Two such mares that had formed an AHF in synchrony with the ovulations in the donor mares were successfully used as recipients. Each received a daily oral administration of 0.088 mg/kg allyl-trenbolone (Regumate: Intervet UK Ltd., Buckinghamshire, UK) during the first 150 days of gestation and both carried their pregnancies to term.

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