First record of abortion of single co-twin in an Arabian mare and continuity of the other to full term and birth

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Abstract

Twin pregnancies in Arabian horses is a major cause of abortion. In all cases when the abortion occurs, it occurs for both fetuses. Unusually in the case described here, which we believe has not previously been documented in Arabian horses, one fetus aborted in undersized and representing an age of 4 months. The other fetus completed the gestation and reached full term followed by birth. The authors postulated that one fetus underwent a stage of diapause like marsupials in a blastocyst stage then re-grew again, followed by abortion. The parentage analysis confirmed that both feti are derived from the same dam and sire stallion.

Case Report:

First record of abortion of single co-twin in an Arabian mare and continuity of the other to full term and birth

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Summary

Twin pregnancies in Arabian horses is a major cause of abortion. In all cases when the abortion occurs, it occurs for both fetuses. Unusually in the case described here, which we believe has not previously been documented in Arabian horses, one fetus aborted in undersized and representing an age of 4 months. The other fetus completed the gestation and reached full term followed by birth. The authors postulated that one fetus underwent a stage of diapause like marsupials in a blastocyst stage then re-grew again, followed by abortion. The parentage analysis confirmed that both feti are derived from the same dam and sire stallion.

Key words:

Equine twins, abortion, blastocyst capsule, diapause.

Introduction

Early pregnancy in the mare is a fascinating period that comprises numerous profound developmental changes and events, many of which are characteristic to the horse. (Stout,2009). At ovulation, the oocyte is released into the relatively small oviductal fimbria and dropped into the ampullary region where, if capacitated sperm are present, fertilization will take place (Boyle et al., 1987 and Hunter 2005). Once fertilized, the newly formed "zygote" embarks on a series of regular cell divisions, such that it reaches the two-cell stage within 24 hours, four to six cells within 48 hours, and by 72 hours contains eight to ten cells (Bezard et al., 1989). Between days 4 and 5, a "morula" is formed, and the as yet undifferentiated cells "compact" into a homogenous ball until late on day 5 or early on day 6 when the embryo starts to develop into a "blastocyst" (i.e., to form a central cavity while the constituent cells differentiate visibly into either inner cell mass or trophectoderm). At around the same time, day 6–7 after ovulation, the embryo finally enters the uterus. (Freeman et al., 1991; Battut et al., 1997).

A blastocyst capsule is one of the defining characteristics of pre-fixation horse conceptus development. Although the exact functions of the capsule are not known, it's tough, elastic nature almost certainly allows it to provide mechanical protection (Stout and Allen, 2001).

The most well-known historical and important cause of abortion in thoroughbreds was twin formation (Acland, 1987, 1993). Twins are usually originating from diestrus ovulation. The outcome for the majority of twin pregnancies is early fetal resorption or loss, late term abortions, or the birth of small growth retarded foals. Mares aborting twins in late gestation frequently have dystocia, damaging effect on their reproductive tracts, and are difficult to rebreed. If foals are born alive they are frequently small, showing the effects of intrauterine growth retardation, and have a very poor survival rate, with many essential expensive complicated critical care (McKinnon, 2011).

There is a phenomenon called an embryonic diapause in mammals which is a period of developmental arrest, in which the blastocyst is maintained in a dormant state for an extended period of time. Over 100 species of mammals undergo embryonic diapause (Renfree, Calaby 1981 and Renfree, Shaw 2000), including the mouse and around 30 species of marsupials. In diapausing mammals, the entry into diapause is controlled by signals including the suckling stimulus, the change in daylength or the availability of nutrition (Flint et al., 1981).

Case history

A 7 years old purebred Egyptian Arabian mare came to our clinic (Derbala Equine Clinic) for breeding and she became pregnant. The mare was examined three times at 15 days, 30 days and 45 days of pregnancy. Following these examinations, she was sent to a nearby farm that contains only foals and stallions. On 9 months of pregnancy the owner called the clinic telling us that the mare aborted a small foal. The mare and aborted foal admitted to the clinic for examination.

Clinical findings and diagnosis

With physical examination there was bloody discharge on the tail and perineal area. The abdomen was still enlarged. Transrectal scanning was carried out and revealed the presence of a viable fetus of 9 months age determined through estimation of eyeball diameter and the date of insemination, with presence of obvious increase of CTUP (Combined thickness of uterus and placenta) measurement with an average 1.33 cm (Fig. 1-A.), the mare underwent to classical treatment of placentitis. By physical examination of the aborted fetus; it appeared as hairless fetus similar to 4 months pregnancy age aborted fetus (Fig.1-B). A skin biopsy was taken from the aborted fetus with hair samples from the dam and the father stallion then sent to Genome Research Unit for analysis. Then after delivery of the other foal a hair sample was sent to the same lab as well (Fig. 2).

Fig. 1-A. abnormal increased size of combined thickness of uterus and placenta (CTUP) of the residual twin. **Fig. 1-B.**

Fig. 2. The second co-twin foal after birth, the hair sample was taken from the foal for genotyping

Laboratory analysis:

A skin biopsy was received from the aborted fetus with hair samples from the dam, the sire and the other foal for DNA genotyping and parentage verification. Genomic DNA was extracted from the samples using an EZ-10 Spin Genomic DNA Minipreps purification kit following the manufacturer's protocol. A total of 12 microsatellite markers (AHT4, AHT5, ASB17, ASB23, HMS6, HMS7, HTG4, VHL20, HMS3, ASB2, HTG10, HMS2) specific to Equus caballus were used in this study. All markers are included in the panel recommended by the International Society for Animal Genetics for diversity studies and parentage verification. The 12 microsatellites are amplified in one multiplex reaction using Stockmarks; horse genotyping kit (Cat. No.: PN4336407 – Applied Biosystem - USA) according to the method described by (Sargious et al., 2014). Fragment sizes of microsatellite alleles were determined using Genetic analyzer 3500 (Applied Biosystem-USA) with the aid of Liz standard. The data obtained is further analyzed using Gene Mapper V 4.1 software (Applied Biosystem, USA). The Proposed nomenclature for the 12 equine short tandem repeat loci investigated is based on the number of repeat units and is adopted from the recommendation of International Society of Forensic Genetics (ISFG) for the nomenclature of human STRs (Bozzini et al., 1996). Global standardization of molecular marker profiles and their use within animal parentage verification is currently governed by various institutes, inclusive of ISAG (https://www.isag.us/)

Table (1):

ID	VHL20	HTG4	AHT4	HMS7	AHT5	HMS6	ASB2	HTG10	ASB17	HMS3	HMS2	ASB23
Sire	R	M	JK	L	MN	LM	OQ	LR	MR	M	LM	I
Dam	LN	K	HO	K	KM	L	K	L	MQ	M	M	I
Aborted fetus	NR	KM	JO	KL	KN	L	KQ	L	MR	M	LM	I
Live Foal	LR	KM	HJ	KL	KM	LM	KQ	L	M	M	M	I

The parentage verification was qualified by the compatibility of 12 microsatellite markers according to Mendelian fashion.

Discussion

Typical methods employed to perform parentage verification apply molecular technology to test simple Mendelian inheritance principles between offspring, paternal and maternal candidates (Jones et al. 2010). This concept of molecular based parentage verification is currently applied within a range of diploid species including the equine species and perform as a key powerful tool to ensure integrity of pedigree recording, when testing offspring against nominated sires and dams (Marklund et al. 1994, Binns et al. 1995, ISAG 2012).

The Horse Genetic Committee of the last virtual international society of Animal Genetics (ISAG) Conference (2021) presented 12 microsatellite markers (AHT4, AHT5, ASB2, ASB17, ASB23, HMS2, HMS3, HMS6, HMS7, HTG4, HTG10 and VHL20) as international minimum standard microsatellite marker system. Our result in this case agreed that microsatellite DNA typing of the aborted fetus and another live foal are compatible with the 12 microsatellite markers of the dam and sire according to Mendelian fashion (Myres et al., 2009). The data presented in our study is providing the effective tool for confirming parentage.

This article presents a record of abortion of a single co-twin fetus in a pregnant mare and the other fetus completed the gestation period until full term followed by normal birth. As aforementioned the results of genetic analysis for parentage confirmed that the two feti are co-twin from the same sire and dam. The most important cause of abortion in Thoroughbreds is twin as described by Acland (1987, 1993). Twins

also lead to dystocia, harmful effect on reproductive tract and intrauterine growth retardation (McKinnon, 2011), While in this case discussed in the article, the abortion taken place for a single fetus in an age smaller than the other co-twin (single abortion) and continuity of the other fetus till birth. In our opinion, the most reliable explanation is embryonic diapause to the singleton for a time period that may reach few months, especially this phenomenon is recorded in some mammals such as marsupials (Renfree and Calaby 1981, Renfree and Shaw 2000 and Flint et al , 1981), in which the embryo reaches blastocyst stage then enters a dormant stage, moreover, the blastocyst capsule which is tough and elastic in nature almost provides mechanical protection inside the uterus (Stout and Allen, 2001).

CONFLICT OF INTEREST

No conflicts of interest have been declared.

ETHICS STATEMENT

Not applicable to this case report.

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