CSIRO PUBLISHING

SPECIAL ISSUE | REVIEW
https://doi.org/10.1071/RD22239

Reproduction, Fertility and Development

Lethal variants of equine pregnancy: is it the placenta or foetus leading the conceptus in the wrong direction?

Charlotte A. Shilton[A], Anne Kahler[A], Jessica M. Roach[A], Terje Raudsepp[B] and Amanda M. de Mestre[A,*]

For full list of author affiliations and declarations see end of paper

***Correspondence to:**
Amanda M. de Mestre
Department of Comparative Biomedical Sciences, The Royal Veterinary College, Hawkshead Lane, Herts, AL9 7TA, UK
Email: ademestre@rvc.ac.uk

Published: 7 November 2022

Cite this:
Shilton CA *et al.* (2023)
Reproduction, Fertility and Development, **35**(1–2), 51–69.
doi:10.1071/RD22239

ABSTRACT

Embryonic and foetal loss remain one of the greatest challenges in equine reproductive health with 5–10% of established day 15 pregnancies and a further 5–10% of day 70 pregnancies failing to produce a viable foal. The underlying reason for these losses is variable but ultimately most cases will be attributed to pathologies of the environment of the developing embryo and later foetus, or a defect intrinsic to the embryo itself that leads to lethality at any stage of gestation right up to birth. Historically, much research has focused on the maternal endometrium, endocrine and immune responses in pregnancy and pregnancy loss, as well as infectious agents such as pathogens, and until recently very little was known about the both small and large genetic variants associated with reduced foetal viability in the horse. In this review, we first introduce key aspects of equine placental and foetal development. We then discuss incidence, risk factors and causes of pregnancy loss, with the latter focusing on genetic variants described to date that can impact equine foetal viability.

Keywords: abortion, aneuploidy, chromosome, CNV, early pregnancy loss, embryology, equine, fetus, genetics, horse, mare, miscarriage, monosomy, placenta, pregnancy complications, pregnancy loss, SNPs, translocation, trisomy, trophoblast.

Introduction

It is believed that the ancestors of the modern horse were domesticated across the Western Eurasian steppe (modern day Northern Kazakhstan) around 4000 years ago (Outram *et al.* 2009). Unlike other prey animals that were domesticated for food purposes (cattle, sheep, pigs etc.), horses were more likely domesticated primarily for transportation, farming, and warfare purposes. Through the mating of related individuals with prized phenotypic traits, the process of domestication of horses has led to the modern horse possessing speed, agility and strength frequently on display on the arena, cross country course or racetrack. The majority of horse breeders therefore select mare and stallion combinations based on prior performance and musculoskeletal and cardiovascular traits. With reproductive characteristics playing second fiddle, it is unsurprising that the modern horse genome contains an excess of deleterious mutations compared with their wild ancestors [reviewed by (Raudsepp *et al.* 2019)].

Reproductive performance studies over the decades point to pregnancy loss as one of the greatest challenges to breeding operations. Due to the seasonal oestrous cycle and the programmed lifespan of endometrial cups in the mare, even pregnancies lost in the first 2 months of gestation can significantly disrupt breeding efficiency. A US study of Thoroughbred (TB) breeding showed the importance of mares producing a foal in six out of seven breeding seasons (Bosh *et al.* 2009*a*). Even relatively wealthy sports such as Thoroughbred racing, breeding is not devoid of economic challenges. Indeed, a more recent economic impact study in the UK reported that whilst the United Kingdom (UK) TB breeding industry alone contributes £427 million to the economy and over 19 000 jobs, the profitability of the sport to breeders has declined, with over 66% of operations now operating at a loss (PriceWaterhouseCoopers LLP 2018). For more endangered breeds, reproductive efficiency is critical to their survival (Orlando and Librado 2019).

Pregnancy can fail at any stage from conception to parturition. The underlying reasons for these losses are variable but ultimately most cases will be attributed to a primary pathology of either the mare (such as endometrial health, endocrine function, immunopathology and oocyte characteristics) or the embryo/foeto-placental unit (inherited via the germline from the stallion or mare or acquired during development). In both cases, defects could be intrinsic to the tissue or alternatively the response of that tissue to extrinsic factors such as pathogens, nutrients and environmental changes and/or contaminants. Whilst previous research has focused on both intrinsic and extrinsic factors that impact the environment in which the embryo develops, surprisingly little is known about defects intrinsic to the embryo itself. Both large and small genetic variants intrinsic in the conceptual tissues are commonly associated with miscarriage in women but to recently, little was known about their role in foetal viability in the horse. The aim of this review is therefore to focus on identifying all known genetic causes of pregnancy loss in the mare. We start by introducing the foundations of equine placental and foetal development essential for the delivery of a healthy offspring. We then move on to review the known causes and risk factors for pregnancy loss at the pregnancy, mare, stallion and environmental level with a focus on those that may relate to genetic conditions. In part three, we outline specific structural variants and deleterious mutations associated with embryonic or foetal lethality and discuss their origins and possible explanations for phenotypic heterogeneity.

Normal foetal and placental development

Equine pregnancy lasts approximately 340 days (or 11 months) during which time, the foetus undergoes coordinated growth and development, allowing delivery of a new-born foal capable of running alongside its mother within just a few hours of birth (Allen and Wilsher 2009). There are two main phases of pregnancy: the early gestation period is approximately 2 months and encompasses the transformation of the simple single cell zygote into a foeto-placental unit comprised of fully formed foetus with a full cohort of organs and a chorioallantoic placenta that has firm contact with the endometrium. The rest of pregnancy thereafter is taken up with a period of continued, rapid and co-ordinated expansion and growth of the placenta and foetus.

Early embryonic development

Approximately 5.5–6 days following ovulation, the compact morula (or early blastocyst) is selectively transported through the uterine-tubal junction to the uterus, where it migrates between the two uterine horns and body for approximately 10 further days. This phase of mobility is unique in the mare and believed to contribute an anti-luteolytic signal to the endometrial surface, although its precise role remains undetermined (Swegen 2021). Conceptus mobility is facilitated by propulsive uterine contractions, probably resulting from the local secretion of prostaglandin, at least partly by the embryo itself (Stout and Allen 2002), and the spherical shape of the embryo and its glycoprotein capsule. The glycoprotein capsule forms underneath the pre-existing zona pellucida, growing to approximately one micron thick between days 6 and 8. The zona pellucida is shed by day 8, revealing the capsule (Flood *et al.* 1982), believed to protect the early conceptus from the powerful contractions of the maternal uterine environment (Allen and Wilsher 2009). Gradual disintegration of the capsule begins around day 21 (Oriol *et al.* 1993). The capsule is essential for equine embryo development as removal from day 6 embryos results in embryonic death (Stout *et al.* 2005). Puncturing of the capsule can also prove fatal, depending on the size of the hole (Wilsher *et al.* 2020): *in vitro* puncturing of day 7 or 8 embryos with an acupuncture needle of approximately 100 μm diameter led to no pregnancies from the 10 embryos transferred to recipient mares. Puncture of day-7 or day-8 embryos with far smaller needles – micromanipulator biopsy pipette (~30 μm diameter) or microneedle (<1 μm tip) – resulted in pregnancy rates of 75% and 67% respectively, indicating a small allowance for repair mechanisms by the capsule. One potential protective function of the capsule is in aiding the expansion of the embryo (from morula stage to blastocyst) caused by an influx of fluid. This blastocoel fluid has recently been shown to contain several important proteins, including equine pregnancy-associated glycoprotein (PAG) and FK-506-binding-protein-4 (FKBP4) (Swegen *et al.* 2017).

Placental development

The equine placenta is a remarkable organ, growing rapidly and constantly differentiating (Chavatte-Palmer *et al.* 2022). The entire conceptus is only 26 mm at day 20, yet by term, the placenta fills the entirety of the uterine body and the two horns and weighs around 3–5 kg. Equine placentae are epitheliochorial with six layers separating maternal and foetal blood streams (Allen and Wilsher 2009). Formation of extraembryonic membranes is critical to the survival of the conceptus. Prior to the development of the mature epitheliochorial placenta, the equine conceptus relies on a yolk sac placenta. This transitory, primitive structure is essential for supporting the early embryos reliance on histotrophic nutrients dynamically regulated and secreted from the endometrial glands (Bastos *et al.* 2019). The expanded blastocyst is composed of inner cell mass (ICM), which will develop into the embryo, and the trophectoderm, which forms the extra-embryonic cell linages, and gives rise to the feto-placental unit.

By day 10, the ICM has differentiated into two cell layers, forming the bilaminar embryonic disc. The inner layer, the

endoderm, grows and expands to line the trophoblast layer to eventually enclose the yolk sac. On day 12, with the onset of formation of the primitive streak, the equine embryo measures approximately 12–14 mm in diameter. At day 14, now 17–22 mm, the pregnancy is usually diagnosed via transrectal ultrasound in stud medicine practice by the presence of an embryonic vesicle measuring 17–22 mm (Gaivão *et al.* 2014). Between days 30 and 40, the yolk sac ceases to expand and incorporates into the umbilical cord between days 40 and 50. Coinciding with the regression of the yolk sac is the rapid expansion of the allantochorial placenta that takes over nutrient, energy, and oxygen supply to the foetus, although histotroph secretion continues throughout gestation.

By day 20, the allantois (a fluid filled sac that collects waste from the embryo) appears from the embryonic hindgut, and begins to expand and surround the spherical conceptus (Allen and Wilsher 2009). During this time the capsule begins to thin and rupture, separating it from the conceptus (Oriol *et al.* 1993; Allen and Wilsher 2009). At day 24, the allantois meets the chorionic membrane and fuses to form the allantochorion (Ginther 1998). At day 28, the allantochorion occupies approximately one-third of the conceptus. By day 35, the allantochorion will surround almost the entirety of the conceptus, save for a small section at the embryonic pole called the bilaminar omphalopleure, the future site of umbilical cord attachment.

The primitive chorionic girdle, first identifiable microscopically at day 28, is a structure made of tall, columnar trophoblast cells, which becomes visible to the eye by day 34 reviewed extensively elsewhere (Allen and Wilsher 2009; Antczak *et al.* 2013). During the maturation process, the trophoblast layer of the primitive girdle undergoes rapid cell division resulting in finger-like folds. At day 35, it is mature and compromised of terminally differentiated binucleate, elongated columnar trophoblast cells (Allen and Wilsher 2009). Between days 35 and 37, chorionic girdle trophoblast cells invade into the maternal endometrium via the endometrial glands and begin endometrial cup formation (Allen and Wilsher 2009). Endometrial cups are the source of equine chorionic-gonadotrophin (eCG) which reaches peak serum concentrations between days 70 and 80 then declines as the cups regress until approximately day 120 (Wilsher and Allen 2011). The endometrial cups, and the secretion of eCG, are the primary hurdle preventing a mare from becoming pregnant again in the same season following a pregnancy loss (Crabtree *et al.* 2012).

The epitheliochorial placenta does not invade into the maternal endometrium to the same degree as a haemochorial placenta, therefore a far greater surface area of contact is required to support the developing foetus. The surface area of contact between the foetal allantochorion and maternal endometrium is estimated to be 38–50 m^2 in the TB mare, which is affected by numerous maternal factors reviewed by others (Derisoud *et al.* 2021). As a result, primiparous mares produce lighter and smaller foals and placentae with a decrease foal to placenta surface ratio when compared with multiparous mares (Robles *et al.* 2018). In the mare, the surface area is maximised by both having contact across the entire exterior of the conceptus (termed diffuse) and also through the development of microcotyledons, which are small highly folded areas of contact. From day 40, chorionic villi begin to extend into the maternal endometrium, forming a microvillous attachment with capillary beds rapidly developing on both the maternal and foetal sides (Allen and Wilsher 2009). Microvilli continue to fold, branch, and elongate to give rise to the mature microcotyledons by day 150, with the villi within continuing to lengthen and branch throughout the remainder of gestation (Macdonald *et al.* 2000).

Foetal development and growth

The first signs of an embryo proper are visible by days 12–14 post ovulation with the visualisation of the primitive streak, the earliest stage of neural tube formation, with rudimentary optical vesicles visible on day 15 (Franciolli *et al.* 2011) (Fig. 1). The embryonic circulation is established around day 18 (Gaivão *et al.* 2014), with the cardiac prominence first visible at day 19 and a primitive heartbeat detectable by ultrasonography around or shortly after that time (Franciolli *et al.* 2011). By day 25, the embryonic skin begins to lose its transparency and limb buds are visible (Barreto *et al.* 2016), the genital tubercle (primitive reproductive tract) can be distinguished, and the tail begins developing (Franciolli *et al.* 2011). Hoof formation is evident by day 27 (Franciolli *et al.* 2011), while the primitive skull (cartilaginous chondocranium) is first visualised at day 30, then calcifies into bone (osteocranium) by day 35 (Barreto *et al.* 2016). Over the period of 30–65 days post ovulation, the muscles of the fore and hind limbs develop through the differentiation of mesenchyme into muscle fibres (Barreto *et al.* 2016). During this time, the tongue, oesophagus, and intestine develop (Rodrigues *et al.* 2014). Development of the lungs, trachea, and thoracic oesophagus is first visible by day 34 (Franciolli *et al.* 2011) and by day 36 the trachea and lungs have developed and the diaphragm is identifiable. Pigmentation of the retina is first observed at day 30 post ovulation (Franciolli *et al.* 2011) at the same time the heart has completely formed. The embryo proper transitions to being termed a foetus at day 36, reflecting the development of these key organs (Terminology Working Party of the Third International Equine Reproduction Symposium held in Sydney Australia 1982; Allen and Wilsher 2009). Therefore throughout this review, we will refer to an embryo proper up to 36 days and thereafter the foetus, including if encompassing both periods.

While foetal sex identification is possible by day 47 when the external reproductive organs are first visible (Franciolli *et al.* 2011), clinically it is usually performed shortly after, between days 55 and 75, or even later gestational stages

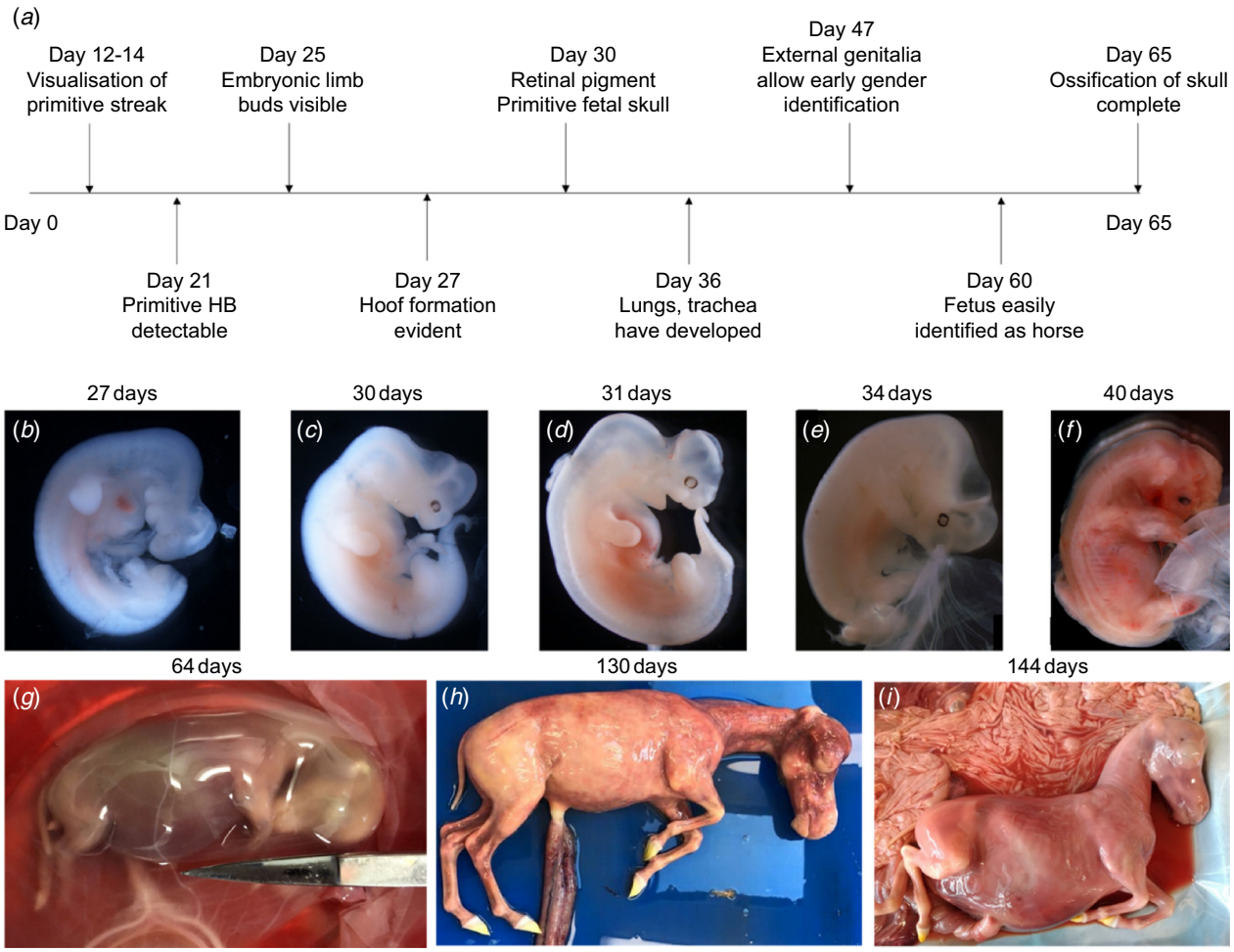

Fig. 1. Early foetal development of the horse. *In utero* development of the embryo/foetus during the first 144 days post ovulation, emphasising the rapid growth and development during the first 4 months. Summary of key developmental events in the transition from embryo to fetus (*a*). Demonstrated in images of fetuses obtained from pregnancies clinically normal at the time of termination or death of the mare showing limb buds (day 27, *b*), retinal pigment (day 30, 31, *c*–*d*), curving and early limb segmentation (day 34, 40, e–*f*), fetus identified as horse with hoof formation clearly evident (day 64–144, *g*–*i*). Images Equine Pregnancy Laboratory.

(Resende *et al.* 2014). Differentiation of the skull begins around day 55, with ossification completed by day 65 (Barreto *et al.* 2016). The foetus undergoes rapid growth during mid–late gestation, along with further cardiac, pulmonary and gonad development (Fowden *et al.* 2020). Foetal size and growth rate may be abnormal in failed or failing pregnancies, with intrauterine growth restriction described in foetuses obtained from clinical cases of pregnancy loss in mares as early as 28–46 days (Kahler *et al.* 2021). Morphologically these foetuses had specific (i.e. failure of neural tube closure) and non-specific abnormalities of the neural tissues and presence of distinct bilateral subcutaneous haemorrhage (Fig. 2). Although this does not reveal an aetiology for the lethality, genetic causes of pregnancy loss may be suspected, similar to associations between chromosomal and foetal morphological anomalies in humans (Byrne *et al.* 1985, 1986; Ursell *et al.* 1985; Kasarskis *et al.* 1998).

Incidence of pregnancy loss

Pregnancy loss can occur anytime from fertilisation to parturition, although very little is reported on losses that occur in the 2 weeks immediately prior to clinical detection in the mare. Whilst miscarriage is the term most commonly used to describe embryonic or foetal death in women, phrases employed in the mare are less standardised. Early pregnancy loss (EPL) is commonly used to encompass losses during the combined embryonic and early foetal stages up to approximately 2 months. Overlapping with this period, early embryonic death (EED) is used to refer to losses during the embryonic stage (prior to 36 days gestation), and early foetal death/loss (EFD/EFL) refers to losses between 40 and 150 days (Morehead *et al.* 2002). After this point, universally any losses up to 300 days are considered to be abortions and thereafter stillbirths (Roach *et al.* 2021).

(*a*)

Clinical EPL samples, submitted between 2013 and 2022:	
No. of EL submissions	143
Gestational age, when a pathology was first noted:	
Youngest conceptus	12 days
Oldest conceptus	67 days
<35 days:	42
35–46 days:	62
>46 days:	20

(*b*)

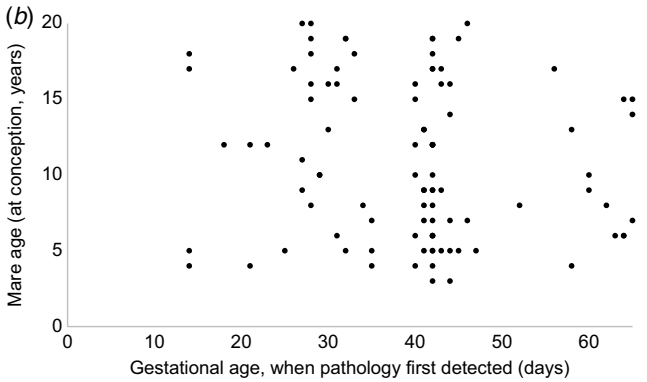

Fig. 2. Characteristics of clinical early pregnancy loss (EPL) abortus material submitted to the Equine Pregnancy Laboratory between 2013 and 2022 (*a*) and mapped based on maternal age at conception and gestational age (days) when pathology was first noted by the veterinary surgeon (*b*).

In practice, research studies often find it difficult to distinguish between stillbirth and early neonatal death through records and will therefore use perinatal death as a phenotype to reflect an inability to accurately pinpoint the time of death. This variation in terms and timing can make comparison of studies challenging and would benefit from standardisation.

The incidence of pregnancy loss has been regularly reported globally over decades (Table 1). While the exact figures vary by population and methodology, collectively it is clear that the early gestation period is the most likely stage when pregnancies fail, mirroring that seen in other species. Further, these figures likely underestimate the true scale of early pregnancy loss as they do not account for losses prior to clinical detection. One study that carefully monitored pregnancies prior to 145 days found that approximately 19% of mare pregnancies were lost between 11 and 15 days gestation and then a further 6% were lost between 15 and 40 days (Ginther 1985). Recently, power doppler has been trialled to allow pregnancy detection in mares 7–8 days post ovulation (Nieto-Olmedo *et al.* 2020) providing hope that we can study pre-clinical embryonic losses more closely in the future. Over the last decade the Equine Pregnancy Laboratory has received conceptus material from clinical cases of early pregnancy loss ranging from 12 to 67 days of gestation (Figs 2, 3), reflecting the gestational age range of EPLs that can practically be isolated to study. There is clear bias in numbers towards EPLs that fail after 28 days compared to what might be expected from clinical monitoring of mares (Fig. 2), showing just how hard it is to perform research on EPLs that fail in the first 4 weeks of gestation.

Rick factors for pregnancy loss

Whilst risk factors alone cannot inform us of the underlying cause of pregnancy loss, epidemiological studies still play an important role when collectively building evidence of cause of foetal death particularly for those associated cases with congenital foetal and genetic abnormalities (Reddy *et al.* 2009). In order to investigate risk factors for pregnancy loss, studies have used varying phenotypes including clinical confirmation of pregnancy (conception and pregnancy losses in the first 2 weeks of gestation) (Allen and Wilsher 2012; Hanlon *et al.* 2012; Lane *et al.* 2016), EPL (including losses from 15 days to 42, or 65 days) (Nath *et al.* 2010; de Mestre *et al.* 2019) and foetal losses from day 35 to foaling (early pregnancy, abortion and stillbirths) (Miyakoshi *et al.* 2012). They also include a mixture of univariable and multivariable analysis (Allen *et al.* 2007; Bosh *et al.* 2009*b*). Very few studies have explored risk factors specific to pregnancy loss after the first 2 months, and none, to the authors' knowledge, have looked at equine abortion or stillbirth as overt phenotypes using multivariable models. The pathophysiology of pregnancy loss varies significantly depending on the stage of gestation. Whilst there may be general risk factors for pregnancy loss throughout gestation, given the differing underlying causes, it is important to consider early and later term losses separately if we are to use the evidence to better understand causes of pregnancy loss. Most work has focused on identifying risk factors for losses in the first 2 months of gestation, the period with the greatest contribution to reproductive inefficiency and the poorest rate of diagnosed cause. It is also the period when genetic variants are most likely to contribute to lethality, so will be the focus on discussion below. Variables found to modify the risk of EPL fall under pregnancy, mare, stallion, or environmental/external factors.

Pregnancy level risk factors

A direct comparison of breeding methods has found natural mating, artificial insemination, as well as artificial insemination, embryo flush followed by transfer all have a similarly low incidence of early pregnancy loss (4.5–6%) when compared to pregnancies established with cryopreserved *in vitro* generated embryos following ovum pick up, intracytoplasmic sperm injection (ISCI) and embryo transfer (13%) (Cuervo-Arango *et al.* 2019). Further, 26% of *in vitro* produced (IVP) pregnancy losses appear to be associated with a higher proportion of anembryonic vesicles (26% of losses) (Cuervo-Arango *et al.* 2019), compared with just

Table 1. Incidences of global equine pregnancy loss reported (1980–2020) from natural mating.

Citation	Breed	Year	*N* (pregnancies or mares as indicated)	GA (days)	PL (%)
Chevalier and Palmer (1982)	Trotters, draught, ponies	1980–1981	4688 mares	23–43	5.3
Villahoz *et al.* (1985)	Mixed horses	1982–1983	354	15–50	17.3
Ginther (1985)	Horse and Pony	–	100 mares	11–15	19.0
				15–40	4.0
	Quarter horses		804 mares	18–36	8.6
Bain (1969)	TB (Australia)	1957–1962	2562 pregnancies	'Early'	13.5
				'Late'	3.8
Irwin (1975)	Standard and TB	–	487 mares	20–50	5.5
Simpson *et al.* (1982)	TB (UK)	1981	–	20–45	5.0
Morley and Townsend (1997)	TB (Canada)	1988	990 pregnancies	Whole gestation	16.0
Morris and Allen (2002)	TB (UK)	1998	1145 pregnancies	15–35	10.4
				>35	7.0
Hemberg *et al.* (2004)	TB (Sweden)	1997–2001	430 mares	Whole gestation	12.5
Yang and Cho (2007)	TB (South Korea)	2001–2003	376 pregnancies	15–45 days	12.2
Allen *et al.* (2007)	Flatrace TB (UK)	2002	2321 mares	15–42	7.2
				>42	6.3
	National Hunt TB (UK)		1052 mares	15–42	8.0
				>42	8.2
Bosh *et al.* (2009*b*)	TB (USA)	2004	931 pregnancies	15–40	8.9
				>40	12.9
Sharma *et al.* (2010)	TB (India)	1998–2005	253 mares	16–39	9.9
				40–90	3.1
				91–198	4.7
				>199	2.8
Nath *et al.* (2010)	TB (Australia)	1990–2001	4210 pregnancies	13–45	7.1
	TB (Australia)		1837 pregnancies		7.5
Miyakoshi *et al.* (2012)	TB (Japan)	2007–2009	1476 pregnancies	17–35	5.8
			843 pregnancies	>35	8.7
Hanlon *et al.* (2012)	TB (New Zealand)	2006–2008	1775 pregnancies	14–42	5.5
			1704 pregnancies	>42	3.0
Rose *et al.* (2018)	TB (UK)	2013–2014	2246 pregnancies	15–65	8.0
				>65	4.8
Roach *et al.* (2021)	TB (UK)	2013–2017	3586 pregnancies	70–300	4.0

TB, Thoroughbred; *n*, sample size (mares or pregnancies); PL, pregnancy loss; GA, gestational age; –, not reported black line separates studies on Thoroughbred mares (below line) from other breeds (above line).

7/55 (13%) in non-IVP EPLs (Shilton *et al.* 2020). This is even more pronounced if embryo diameter at the time of transfer is considered: with 45% of embryos less than 300 μm at the time of transfer developing into an anembryonic vesicle (Claes *et al.* 2019; Cuervo-Arango *et al.* 2019). The exact underlying reason for anembryonic vesicles is not fully understood, with just one associated with autosomal aneuploidy (Shilton *et al.* 2020). The size of the embryo is also associated with modified risk of EPL for both IVP (Cuervo-Arango *et al.* 2019) and *in vivo* produced embryos (de Mestre *et al.* 2019). The odds of EPL is reduced by 0.26 for each 10 mm increase in embryo size at first scan in TB mares (de Mestre *et al.* 2019).

The presence of two or more conceptuses is associated with a high risk of early and late pregnancy loss (Jeffcott and Whitwell 1973). In the mare, these multiples are nearly always dizygotic, with monozygotic multiple pregnancies reported in the literature in just a handful of *in vivo* generated embryos (Meadows *et al.* 1995; Govaere *et al.* 2009) and 1.6% of IVP embryos (Dijkstra *et al.* 2020).

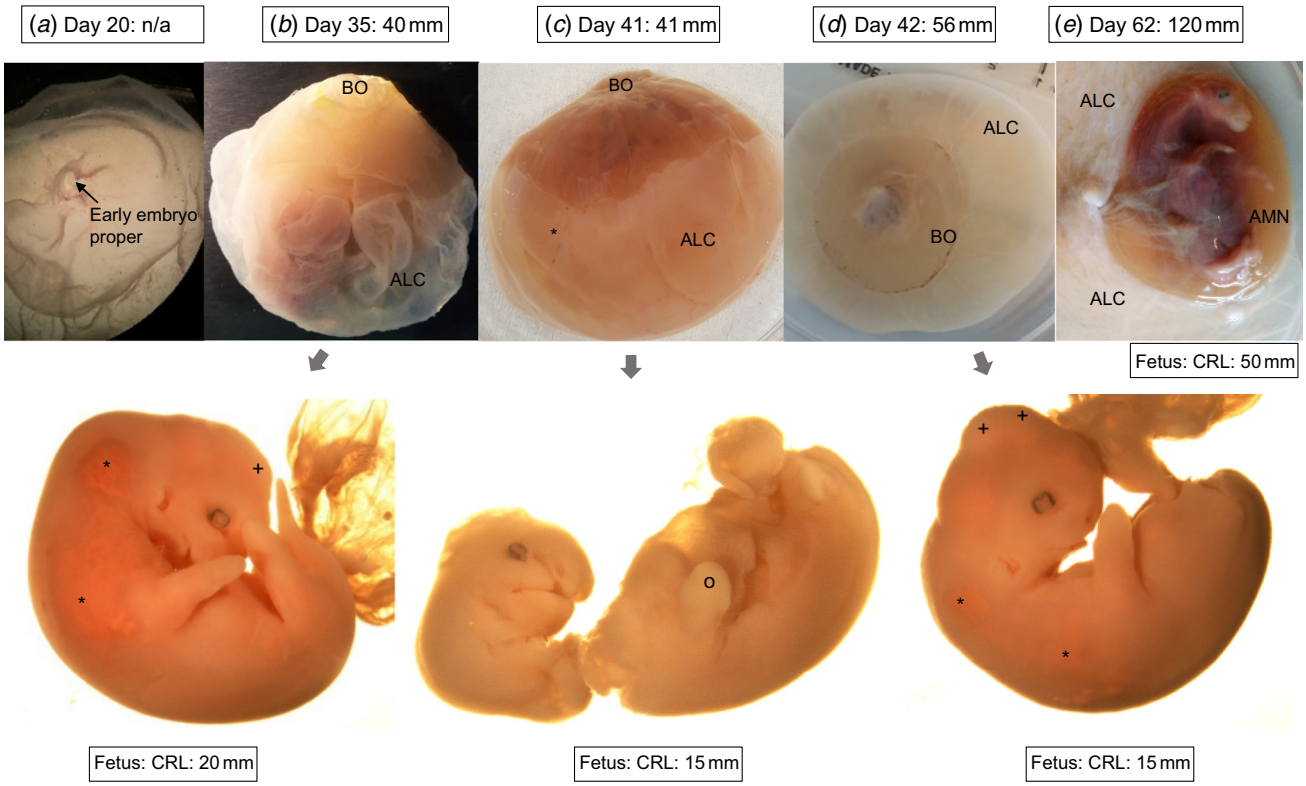

Fig. 3. Representative samples of equine EPLs, submitted to the Equine Pregnancy Laboratory. Top: Conceptuses during their initial assessment and processing on the day of arrival in the laboratory. Please note the appearance of the early foetal membranes, e.g. 'translucent' (*a*) or 'thickened and cloudy' (*b*, *d*); and the absence (*b*) or presence [* in (*c*)] of blood vessels. Diameter of the allantochorion (ALC) in mm. Bottom: Microscopic images of foetuses from conceptus (*b*), (*c*) and (*d*), respectively. An intact amnion (AMN) with amniotic fluid was appreciable at day 62 (*e*). Crown-rump-length (CRL) in mm. Gross morphological abnormalities include an irregular pattern the CNS surface [+ in (*b*) and (*d*)] and a distinct bilateral dark-red discolouration. This 'red patching' is mostly located within the area of neck, shoulder and cranial thorax of a foetus and presents as localised severe subcutaneous haemorrhage in histology [* in (*b*) and (*d*)]. Please note a severely altered development of the foetal head and face and delayed limb development of the foetus in (*c*), appreciable despite autolytic changes of this sample. The visible right front limb (o) is not subdivided and 'bent towards the midline' as expected for this gestational age and development of foot pads has not occurred.

Although conception of multiple pregnancies may be less relevant to EPL in modern veterinary practice: multiple conceptions are routinely reduced at around day 16 of gestation so only one conceptus is carried to term (Ginther and Griffin 1994). Such a reduction is shown across multiple studies to not increase the risk of EPL for the remaining conceptus (Nath *et al.* 2010). Its impact on abortion and stillbirth is not reported, but current investigations in the Equine Pregnancy Laboratory suggest abortion has distinct risk factors to EPL. Whilst twin reduction has not been associated with an increased risk of EPL (de Mestre *et al.* 2019), flunixin meglumine at time of twin reduction was found to be protective (de Mestre *et al.* 2019).

Mare level risk factors

As in many other mammalian species studied to date (Quenby *et al.* 2021), advancing maternal age is a well-known risk factor for early pregnancy loss in horses (Rose *et al.* 2018; de Mestre *et al.* 2019; Fawcett *et al.* 2021). The pathologies of older mares are extensively reviewed by others (Derisoud *et al.* 2021) and in brief include endometrial pathologies such angiosclerotic changes (Grüninger *et al.* 1998) and lower quality oocytes and subsequently embryos (Rizzo *et al.* 2019, 2020). In an attempt to differentiate between oocyte related issues and uterine related issues of older mares, morphologically normal embryos from cycling donor mares were transferred to either normal or sub-fertile mares (Ball *et al.* 1987). There was no difference in the loss rate between the embryos transferred to the normal (9%) or sub-fertile (11%) mares by day 28 of gestation. The same group undertook another experiment in which embryos from normal mares (aged 5.7 and primi/multiparous) and sub-fertile mares (aged 19.4 with a history of reproductive failure) were transferred into normal fertile recipient mares and monitored until day 14 (Ball *et al.* 1989). A significant difference in the rate of embryonic loss between the normal donors (9%) and the sub-fertile donors (62%) was found,

although only in the very early stages of gestation. These two publications highlight the importance of oocyte quality for early pregnancy outcome and are highly relevant when considering genetic causes of pregnancy loss.

The quality of the endometrial environment has also been associated with increased risk of EPL. Uterine cysts are fluid-filled structures and can be separated into two categories: glandular (located in the connective tissue layer, up to 1 cm diameter) or lymphatic (bulging into the uterine lumen, up to 20 cm diameter) (Stanton *et al.* 2004). Two recent studies using multivariable analysis showed an increased risk of EPL with the presence of cysts (Miyakoshi *et al.* 2012; de Mestre *et al.* 2019) even after maternal age and status had been accounted for in the models. It remains unclear whether the cysts themselves cause EPL and/or rather indicate underlying endometrial pathologies that could cumulate in EPL as reviewed here (Katila and Ferreira-Dias 2022). Mares can be bred on their foal heat, however, there are concerns that this practice may lead to an increased incidence of EPL. Multivariable analysis has shown both no modified risk (de Mestre *et al.* 2019) or an increased risk of EPL with foal heat cover (Miyakoshi *et al.* 2012; Lane *et al.* 2016).

Body condition score (BCS) of the mare is not a factor that has been explored in multivariable modelling of pregnancy losses however univariable studies indicate that it is possibly the change in BCS which is important as opposed to the score itself (Miyakoshi *et al.* 2012). Increasing BCS was found to be associated with a significantly lower PL rates between day 17 and 35 of gestation compared to a constant or decreasing BCS, highlighting the benefits of an increasing plane of nutrition during the early pregnancy period. Conversely, a small study found high-energy diets may increase the risk of EPL in Shetland Ponies (D'Fonseca *et al.* 2021). Nutrition is clearly important for foetal morbidity and mortality (Robles *et al.* 2022) and levels and constituents should be controlled if pregnancy outcome is to be optimised.

Stallion level risk factors

Advancing stallion age is associated with decreased fertility due to declining testicular function, affecting both gametogenesis and hormone production (Turner 2019). Stallion level risk factors though have been more difficult to pinpoint when considering pregnancy loss of populations, with most studies suggesting it is mare not stallion that has the greatest contribution to variability (Hanlon *et al.* 2012; Lane *et al.* 2016; de Mestre *et al.* 2019). When stallion factors do contribute, the impact is more commonly felt on day 14 pregnancy rates and this conception/very early pregnancy loss than on pregnancy losses beyond 15 days. Greater than 21 covers per week is associated with decreased pregnancy rates but not loss of clinically confirmed pregnancies (Lane *et al.* 2016). Age of stallion or individual stallion when included as a random effect have not been found to be significant contributors to EPL risk in multivariable analysis (de Mestre *et al.* 2019). Therefore, whilst genetic variants inherited through the germline via the stallion may still yet contribute to pregnancy loss in the mare, these risk factor studies suggest their contribution is small perhaps as such stallions are quickly removed from the breeding pool. Indeed, when 22 stallions were directly compared just 1/22 had significantly higher odds of EPL compared with a reference stallion (de Mestre *et al.* 2019) consistently with work of others (Allen *et al.* 2007).

External/environmental level risk factors

Whilst therapeutics targeted at the endometrium have increased significantly in use over the last decade, neither the administration, nor type of uterine treatment have been found to be associated with a modified risk of EPL across multiple studies (Allen *et al.* 2007; Lane *et al.* 2016; de Mestre *et al.* 2019). This suggests that endometrial conditions, including endometritis, are mostly being managed effectively at the population level to enable maintenance of the pregnancy to at least 65 days and possibly beyond. Induction of ovulation has been found to reduce the risk of EPL (de Mestre *et al.* 2019), possibly acting via the quality of the ovulated oocyte or early luteal function (Köhne *et al.* 2014). Whilst not epidemiological studies are lacking, it is still clear that the presence or absence of pathogens is key to pregnancy loss (Macleay *et al.* 2022) as demonstrated by the marked geographic variation in reporting of certain pathogens.

Causes of pregnancy loss

Pregnancy loss is a condition that can arise from a vast array of possible causes which vary by gestational age at the time of loss, geographic region, breed and management intensity. Broadly, losses fall into two categories, infectious and non-infectious. A recent scoping review of mid and late gestational losses indicates the intensive interest of the research community in infectious causes of pregnancy loss with studies of placentitis, EHV-1, Chlamydia, Leptospirosis and Nocardioform placentitis dominating the literature of the last three decades (Macleay *et al.* 2022). Equally, once we drill down to individual populations, the picture can look quite different. For example, a cohort study of 3516 pregnancies in a subpopulation of Thoroughbred mares in the UK and Ireland found a very different distribution of causes (Roach *et al.* 2021). None that underwent post-mortem examination were attributed to infectious agents such as Chlamydia, Leptospirosis or Nocardioform placentitis and just 5% of pregnancy losses found to be positive for EHV-1 infection and 5% diagnosed with infectious placentitis, a clear reminder that geography and management practices are key when it comes to understanding pregnancy loss in mares.

As well as dividing causes of pregnancy loss by presence of a pathogen, one could also consider the tissue involved in the pathology. The endometrium is one such tissue that has garnered great attention over the decades due to its key role in nurturing the developing embryo and foetus. Endometritis (Morris *et al.* 2020) and endometrial degeneration (Miyakoshi *et al.* 2012; de Mestre *et al.* 2019; Katila and Ferreira-Dias 2022) have been linked with pregnancy loss in the mare both in early and late gestation. Even in mare populations routinely swabbed as a prerequisite to breed, endometrial bacterial infections can be found around the time of the loss in 5–16% of EPLs (Ricketts *et al.* 2003; Hamstead *et al.* 2012; Rose *et al.* 2018). Similarly, the quality of the endocrinological environment has been a topic of hot debate, in particular when it comes to the importance of progesterone levels for pregnancy maintenance. Progesterone production across equine gestation is complex (Conley and Ball 2019) and its role in pregnancy loss has been controversial for decades (Allen 2001). Luteal insufficiency in early pregnancy is categorically a cause of pregnancy loss but an old but well cited study suggested this was uncommon (Irvine *et al.* 1990). Work over the last decade has questioned this early evidence (Betteridge *et al.* 2018) suggesting a precipitous drop in progesterone occurs more frequently preceding early pregnancy loss than previously thought. Further, more nuanced effects of early luteal progesterone levels on a number of pregnancy related parameters, including day 14 pregnancy rates (Hollinshead *et al.* 2022), endometrial function (Beyer *et al.* 2019), as well as early embryonic and foetal growth and organ development (Willmann *et al.* 2011; Okada *et al.* 2020) suggest much needs to be done to understand the role of progesterone levels in pregnancy loss.

Whilst the endometrial and endocrine environment are clearly important, it is estimated that 50–80% of early pregnancy losses (Hamstead *et al.* 2012) and approximately half of mid to late gestation losses (Roach *et al.* 2021; Macleay *et al.* 2022) have no attributed cause. There are a number of well described genetic variants of human foetuses associated with a phenotype of foetal lethality that account for well over 50% of miscarriages (Chen *et al.* 2021). Whilst congenital abnormalities of the developing equine foetus have been long identified (Giles *et al.* 1993; Hong *et al.* 1993), it has only been relatively recently that specific genetic variants have been linked to lethal congenital abnormalities (Ducro *et al.* 2015; Monthoux *et al.* 2015; Shilton *et al.* 2020). One of the challenges when it comes to congenital defects with or without a genetic cause is proving that the congenital abnormality constitutes a cause of death. Reddy *et al.* (2009) provide a valuable classification system developed for human stillbirth. They state in order for it to be a cause of death, 'there are epidemiologic data demonstrating an excess of intrauterine mortality, the process is rarely seen in liveborn neonates and when the process is seen in liveborn neonates, if frequency results in death or there is biologic plausibility that it can result in death'. Direct application of this definition to equine pregnancy loss is not possible and will inevitably be clouded by euthanasia of individuals for financial reasons or perceived implications on athletic potential. Nevertheless, with refinement it is likely to be useful and worth considering here when looking at genetic variants.

One of the challenges of studying causes of pregnancy loss, in particular in the first 2 months, is access to the abortus material. We have created a biobank of this tissue (Fig. 2), working closely with clinicians in practice who have been instrumental to its success (Rose *et al.* 2016). The biobank is, though, not without its limitations. For example, there are just 12 EPLs that failed prior to 28 days of gestation, suggesting mares suffering EPL within 4 weeks of cover generally expel the tissue prior to or very soon after examination by the veterinary surgeon. EPLs have a mixed presentation, with translucent (Fig. 3*a*) or thickened and cloudy extraembryonic membranes (Fig. 3*b*, *d*), and absence (Fig. 3*b*) or presence of intact vasculature (Fig. 3*c*, *d*) sometimes with exsanguination (Fig. 3*d*). The foetuses associated with these membranes show subcutaneous haemorrhage (Fig. 3*b*), mismatch in developmental features such as limb development when compared gestation age of 41 days and CNS abnormalities (Fig. 3*c*), similar to that reported for earlier foetuses from the bank (Kahler *et al.* 2021). Surprisingly, despite the autolytic nature of these tissues, trophoblast cells and quality DNA have been successfully isolated (Rose *et al.* 2016) from the majority of EPL cases. Attempts to karyotype this material has been challenging (Blue 1981; Haynes and Reisner 1982) as the growth rate of the cells is compromised. DNA based assays are therefore likely to be the most rewarding when assessing the genomes of EPLs.

Genetic causes of pregnancy loss in the mare

Aneuploidy

The most common type of genetic variant associated with pregnancy loss in the mare is aneuploidy (Shilton *et al.* 2020), mirroring that reported in women (Jia *et al.* 2015; Chen *et al.* 2017). Aneuploidy is the gain or loss of an entire chromosome compared to the normal diploid number for that species (Torres *et al.* 2008). There are three categories of aneuploidy type: monosomy (loss of a single chromosome), trisomy (gain of a single chromosome) and nullisomy (loss of two homologous chromosomes). As chromosomes are the carriers of the nuclear genome required for normal cell function, the genomic imbalance caused by aneuploidy are rarely tolerated to term. The best-known exception to this is Down's Syndrome (trisomy 21) in humans, although this variant may also result in pregnancy loss (Hwang *et al.* 2021). Autosomal monosomy ($2n - 1$) has never been reported in a live animal of any species, strong evidence of a foetal

lethal phenotype (Bugno-Poniewierska and Raudsepp 2021). Historically, research on aneuploidy was focused on human pregnancy loss and conditions, with surprisingly little reported in any veterinary species beyond the rare report in cattle (Schmutz *et al.* 1996), pigs (Hornak *et al.* 2012) and horse blastocysts (Rambags *et al.* 2005). The last decade has resulted in increasing uptake in use of veterinary species to study underlying mechanisms of aneuploidy (Rizzo *et al.* 2020). In the horse, both lethal and non-lethal aneuploid types have been reported (Shilton *et al.* 2020; Bugno-Poniewierska and Raudsepp 2021).

Non-lethal autosomal trisomies in horses are rare (Bugno-Poniewierska and Raudsepp 2021). There are just 16 individuals reported in the literature surviving to term (three of which were mosaic) (Power *et al.* 1992). Those identified are often euthanised at a young age (Kubien and Tischner 2002; Bugno *et al.* 2007; Holl *et al.* 2013). The exact phenotypes displayed for non-lethal autosomal aneuploidy are heterogenous. Trisomy of chromosome 28 (65XY,+28) resulted in a colt that displayed reduced growth and cryptorchidism. Trisomy of chromosome 23 (65,XY,+23) in a male Standardbred (Klunder *et al.* 1989) was associated with limb deformities, facial asymmetry, along with other developmental defects. Many of the other reported individuals diagnosed with aneuploidy displayed limb deformities including: angular limb deformities (Bowling and Millon 1990), bilateral contracted tendons of the forelimbs (Buoen *et al.* 1997; Lear *et al.* 1999), bilateral carpal flexural deformity (Zhang *et al.* 1992; Buoen *et al.* 1997), stiff hindlimb gait (Brito *et al.* 2008), and laxity of rear limb flexor tendons (Lear *et al.* 1999). Developmental Orthopaedic Disease is the most common congenital defect reported as a cause of death in thoroughbred foals (Mouncey *et al.* 2022), which begs the question: could some of the cases of neonatal and foal lethality be attributed to underlying trisomies?

Aneuploidy in equine conceptus tissue has been reported in just two studies. An early study by (Rambags *et al.* 2005) identified copy number abnormalities of chromosomes 2 and 4, in 22 *in vivo* and seven *in vitro* produced embryos. As these blastocysts were sacrificed for the study, their viability was not able to be assessed. Nevertheless, this early observation was important in building evidence that aneuploidy was not just a peculiarity of human gametes and embryos. More recently, we reported autosomal aneuploidy in 11/55 (20%) EPLs assessed making it by far the most common pathology of early pregnancy reported to date in well managed breeding mares (Shilton *et al.* 2020). With the exception of trisomy 30, the trisomies and monosomies reported were unique and therefore likely to represent a lethal phenotype, although more time and data is needed to state this with absolute confidence (Shilton *et al.* 2020; Bugno-Poniewierska and Raudsepp 2021).

Aneuploidy types identified to be associated with equine early pregnancy loss are not only unique, but they tend to involve the larger chromosomes and include trisomy 1, 3, 15, 20 and combined chromosomes 23/24. In contrast, aneuploidies of smaller autosomes and sex chromosomes have been reported in live equine births (chromosomes 23, 26, 27, 28, 30, 31, and X). Biology always reveals its nuances with time, but for the moment lethal and non-lethal phenotypes separate out quite clearly, with chromosome sizes of 65 Mb (or 2.8% of the Genome) associated with lethality and those 55 Mb or less (or 2.4% of the Genome) associated with liveborns or a mixed phenotype (Shilton *et al.* 2020; Bugno-Poniewierska and Raudsepp 2021). Though, interestingly, there have been no reports of liveborn equine trisomy 29 (34 Mb), suggesting that it is not only size, but also genetic composition of a chromosome, that matters when it comes to viability (Bugno-Poniewierska and Raudsepp 2021). Monosomies of small (26, 27, 31) chromosomes all lead to foetal demise by 65 days of gestation. It is worth noting that monosomies of some of the large chromosomes are yet to be identified, perhaps suggesting these are lethal prior to clinical detection and may well be revealed once routine testing of equine blastocysts is established.

Aneuploidies of allosomes, the most frequent of which is X monosomy, are more commonly reported in adult horses. Pure X monosomy is usually associated with infertility, and mosaic X monosomy subfertility, extensively reviewed by Bugno-Poniewierska and Raudsepp (2021). A study of 500 randomly selected horses (across 11 breeds) found that of the 10 chromosome anomalies detected, nine involved the sex chromosomes, eight of which were mosaic (Bugno *et al.* 2007). This is not always the case, with reports from other cytogenetic laboratories failing to identify any examples of mosaic X monosomy (Bugno-Poniewierska and Raudsepp 2021). Whether allosome aneuploides also play a role in pregnancy loss is not known. X trisomy has been identified in a case of EPL (Shilton *et al.* 2020). This could be a coincidence and the pregnancy failed due to another cause. Alternatively, as X trisomy is rare in liveborn horses presenting with gonadal dysgenesis, this aneuploidy type may have a mixed phenotypic outcome as has been shown in humans (Skuse *et al.* 2018).

Mosaicism is a feature of the term human placenta (Coorens *et al.* 2021) and with large scale screening of human blastocysts now common place, we know an aneuploidy trophectoderm cell identified in a human blastocyst does not always equate to foetal aneuploidy (West and Everett 2022). Whilst mosaicism is, as discussed above, a feature of adult horses (Bugno *et al.* 2007), examples of it are as yet to be identified in the equine foeto-placental unit. Only a limited number of EPL tissues have been assessed across both compartments with all assessed placental-foetal duos having a matched diploid status in foetal and placental or multiple placental samples (Shilton *et al.* 2020). We have identified additional aneuploid pregnancies in unpublished work in our laboratory but are still yet to find examples of aneuploid mosiacism. Still,

this is likely a reflection of sample size and they may well be revealed in due course, although perhaps not at the frequency as seen in human placentae (West and Everett 2022).

Aneuploidy most often arises during gametogenesis, as a sporadic event of a single gamete. Maternal meiotic errors account for approximately 84% of human trisomy pregnancies, while paternal meiotic errors account for 11% and post-zygotic errors account for the remaining 5% of cases (Mikwar *et al.* 2020). These figures are not available for the mare. The failure to identify mosaicism in the samples screened to date, together with reported high incidence of aneuploidy of equine MII oocytes (56%) from old mares compared with young mares (16%) (Rizzo *et al.* 2020) strongly supports maternal gametogenesis as the primary contributor to aneuploid pregnancies in the mare. Origins in maternal meiotic errors is further supported by other abnormal features of aged equine oocytes, including enhanced chromosome misalignment (Rizzo *et al.* 2019) and weaker centromeric cohesion of *in vitro* matured oocytes (Rizzo *et al.* 2020). A study investigating the effect of *in vitro* maturation process on the rate of aneuploidy (Franciosi *et al.* 2017) found that *in vitro* maturation IVM oocytes were significantly more affected by aneuploidy than *in vivo* matured oocytes (45.5% vs 0%, respectively). Whether this explains the higher rates of early pregnancy loss of IVP embryos (Cuervo-Arango *et al.* 2019) is not known although worthy of consideration.

It has been suggested that the prolonged arrest of equine oocytes from *in utero* foetal development to ovulation contribute to development of aneuploidy in oocytes. In equines, oocytes from older mares (aged ≥14 years) were found to have both a thicker metaphase plate and a reduction in the correct alignment of chromosomes across it during metaphase II (Rizzo *et al.* 2019). Displacement of chromosomes on the metaphase plate has also been shown to be markedly increased in oocytes during the second meiotic division in older women compared with younger women (40–45 years and 20–25 years, respectively (Battaglia *et al.* 1996). Interestingly, the major spindle axis of equine oocytes is significantly increased in those with chromosome misalignment, regardless of mare age (Rizzo *et al.* 2019).

Aneuploidy can also arise through errors in spermatogenesis (Hassold *et al.* 2007). In analysis of sperm from healthy human donors, 1–2% of spermatozoa were found to be aneuploid (Martin 2007). In stallion sperm, advancing paternal age increases aneuploidy of the allosomes but not autosomes (Bugno-Poniewierska *et al.* 2011). This would suggest aneuploidy of stallion gametes in the horse is more likely to contribute to subfertility of the resulting offspring as opposed to lethality. Risk factor studies of equine pregnancy loss support this conclusion, with neither stallion age nor individual stallion found to be risk factors for early pregnancy loss (Hanlon *et al.* 2012; Lane *et al.* 2016; de Mestre *et al.* 2019) or pregnancy loss at any stage of gestation (Lane *et al.* 2016). Whilst genomic structural errors introduced in early embryonic cell division are well described for human and rodent embryos, they are as yet to be described in the horse.

Translocations

Translocations are the breaking and fusing of chromosome segments between non-homologous chromosomes and have been a cornerstone of genetic diversity throughout evolution. Translocations can be broadly separated into two categories: balanced (the entire complement of genetic material is retained in all cells) and unbalanced (an unequal split between daughter cells, resulting in loss of genetic material in some cells). Of the 15 cases of equine translocations reported to date (Table 2), only a single male had an unbalanced autosomal translocation (64,XY,t(4;30),+4p) (Ghosh *et al.* 2020), with the remaining being balanced autosomal or unbalanced allosomal translocations. Thirteen mares all presented with subfertility with six of 13 mares presenting with a specific history of recurrent pregnancy loss. This is because translocations disturb meiosis and gametogenesis, resulting in formation of both genetically balanced (1/3) and unbalanced (2/3) gametes (Raudsepp 2020). The latter, if involved in fertilisation, may result in pregnancy loss, whereas gametes with the balanced form of translocation will pass the defect to liveborn offspring.

Single nucleotide polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs) are one of the most well-known and commonly studied genetic variant types. A change in one of the bases of the codon that results in a change of amino acid is termed a missense mutation which can result in compromised protein function and an altered phenotype dependent on exactly how the protein is impacted (Alberts *et al.* 2015). There are surprisingly few SNPs reported in the equine literature that are associated with lethality *in utero* or immediately following parturition (Table 3). This is likely more a reflection on the limited research in the area and limited availability of abortus tissue, as opposed to an absence of lethal haplotypes in equine foetuses which are more comprehensively described in women.

Congenital hydrocephalus, excessive accumulation of fluid in the brain, has been identified in several horse breeds. Affected foals are either stillborn or euthanised immediately following birth (Ojala and Ala-Huikku 1992; Ferris *et al.* 2011; Pannu and Singh 2014). Next generation sequencing identified a nonsense mutation in β-1,3-*N*-acetylgalactosaminyltransferase 2 (*B3GALNT2*) associated with congenital hydrocephalus in Friesian foals (Ducro *et al.* 2015). While capable of leading to stillbirth and perinatal death, whether this SNP also contributes to EPL or abortion is unexplored. Pathologies of the central nervous system in foetuses from pregnancies lost in the first 6 weeks of gestation are common (Kahler *et al.* 2021), so a heterogenous phenotype can't be ruled out.

Table 2. Summary of 15 equine translocations reported to date and their associated phenotype.

Case	Sex	Age (years)	Breed	Method	Translocation type	Translocation	Phenotype	References
1	F	6	TB	G-/R-band	Nonreciprocal unbalanced	64,X,t(15;X),−Xp,+15	Short/infertile/very stiff gait	Power (1987)
2	F	–	TB	G-/R-band	Reciprocal balanced	64, XX,t(1q;3q)	Phenotypically normal/REEL	Power (1991)
3	M	8	TB	G-/C-band	Tandem balanced	63,XY,t(1;30)	High incidence of EED	Long (1996)
4	F	5	TB	G-band/ Zoo-FISH	Reciprocal balanced	64,XX,t(1;16)(q16;q21.3)	Phenotypically normal/persistent infertility	Lear and Layton (2002)
5	F	–	TB	G-/C-band/ FISH	Nonreciprocal balanced	64,XX,t(1q;21)	Phenotypically normal/subfertility	Lear *et al.* (2008)
6	F	–	TB	G-/C-band/ FISH	Reciprocal balanced	64,XX,t(16q;22q)	Phenotypically normal/REEL	Lear *et al.* (2008)
7	F	–	TB	G-/C-band/ BAC-FISH	Reciprocal balanced	64,XX,t(4p;13p)	Phenotypically normal/mild chronic endometritis/subfertility	Lear *et al.* (2008)
8	M	–	TB	G-band/ BAC-FISH	Nonreciprocal balanced	64,XY,t(5;16)+mar	REEL	Durkin *et al.* (2011)
9	F	13	TB	G-band/ BAC-FISH	Nonreciprocal balanced	64,XX,t(2p;13p)	REEL	Lear *et al.* (2014)
10	F	3	AR	G-band/ BAC-FISH	Nonreciprocal balanced	64,XX,t(4;10)(q21;p15)	Phenotypically normal/REEL	Ghosh *et al.* (2016)
11	F		–	G-band/ BAC-FISH	Reciprocal balanced	64,X,t(1p;Xp)(1q;Xq)	–	Bugno-Poniewierska *et al.* (2018)
12	M	5	Fr	G-/C-band/ BAC-FISH	Reciprocal balanced	64,X,t(13;Y)(pter;qter)	Phenotypically normal/infertility/ azoospermia	Ruiz *et al.* (2019)
13	F	2	TB	G-band/ BAC-FISH	Nonreciprocal unbalanced	63,X,der(X)del(q22)dup(q21q11) t(X;16)(q21;q11)dic(X;16)	Short	Mendoza *et al.* (2020)
14	M	–	WB	G-band / BAC-FISH	Nonreciprocal balanced and unbalanced	64,XY,t(4;30),der(4q) and 64,XY, t(4;30),+4p	Foals with congenital abnormalities	Ghosh *et al.* (2020)
15	M	–	AR (clone)	G-band/ BAC-FISH	Nonreciprocal balanced	64,XY,t(12;25),der(12p)	Azoospermia/small testes	Ghosh *et al.* (2020)

F, female; M, male; TB, Thoroughbred; AR, Arabian; Fr, Friesian; WB, Warmblood; REEL, recurrent early embryo loss; EED, early embryonic death; FISH, fluorescence *in situ* hybridisation; BAC, bacterial artificial chromosome.

Aberrations in the TP53 gene have also been associated with negative pregnancy outcomes in both horses (de Leon *et al.* 2012) and women (Su *et al.* 2011). A study of 105 mares on a single stud farm in Brazil (de Leon *et al.* 2012), found a heterozygote genotype in exon 4 of p53 (nonsense mutation of non-polar proline to positively charged arginine) was associated with increased risk of abortions (OR = 14.5). It should be noted this was a SNP identified in mares not abortus material, so likely acts on the embryonic environment via the endometrium or immune function although this remains to be explored. Altered expression of p53 has been demonstrated in equine endometrial progenitor cells in obsese mares who in turn present with suboptimal endometrial function (Smieszek *et al.* 2022).

Fragile Foal Syndrome (FFS) is a monogenic disorder with Mendelian inheritance of a recessive allele originally described in a Warmblood (Monthoux *et al.* 2015) but more recently also a Thoroughbred (Grillos *et al.* 2022). Foals homozygous for a single missense mutation (c.2032G>A) in procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 (*PLOD1*) either die *in utero* and present as late gestation abortion or are euthanised shortly following delivery (Aurich *et al.* 2019). Post mortem examinations describe dermal lesions and reduction of collagen fibres (Monthoux *et al.* 2015), keratosis-like thickening of the skin, abnormal flexibility of joints, and spinal cord deformities (Aurich *et al.* 2019). The largest cross breed analysis of PLOD1 recessive allele frequency genotyped 3365 novel individuals and found the mutation was present in 20 of the 38 breeds (Reiter *et al.* 2020) suggesting FFS is likely to be diagnosed in further breeds in the future.

A large scale study of 2556 horses (using both internal samples and publically available genotypes) found two SNPs in *Killer cell lectin-like receptor subfamily 49 B* (*LY49B*), one completely absent in the homozygous state and one with reduced homozygous frequency in the adult populations,

Table 3. Equine SNPs identified to be associated with pregnancy loss.

Phenotype	Condition	Gene	References
Mare genome			
Increased risk of abortion	Abortion unspecified	TP53	de Leon *et al.* (2012)
Foetal genome			
Abortion, stillbirth, neonatal death in all reported cases	Fragile Foal Syndrome	*PLOD1*	Monthoux *et al.* (2015), Aurich *et al.* (2019), Dias *et al.* (2019), Bellone *et al.* (2020), Reiter *et al.* (2020), Grillos *et al.* (2022)
Homozygotes lacking in adult population	Unspecified	*LY49B*	Todd *et al.* (2020)
Stillbirth or euthanised as foals	Congenital hydrocephalus	*B3GALNT2*	Ojala and Ala-Huikku (1992), Ferris *et al.* (2011), Sipma *et al.* (2013), Ducro *et al.* (2015), Kolb and Klein (2019)

TP53, tumour protein p53; *PLOD1*, procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1; *LY49B*, *killer cell lectin-like receptor subfamily 49 B*; *B3GALNT2*, β-1,3-*N*-acetylgalactosaminyltransferase.

suggestive of an inherited lethal phenotype (Todd *et al.* 2020). As *LY49B* is expressed in the trophoblast cells during equine placentation (Read *et al.* 2018) aberrations in this gene, could theoretically result in pregnancy loss but the variant is yet to be identified in abortus material from any stage of gestation.

Copy number variation

Copy number variations (CNV) are large deletions or duplications of the genome, over 1 kb in length, naturally occurring during the cell cycle. It has been estimated that 4.8–9.5% of the human genome comprises CNVs (Kloosterman *et al.* 2015; Zarrei *et al.* 2015) compared with approximately 1.3% of the genome in the horse (Ghosh *et al.* 2014). Variation in copy number from wild type can, however, be associated with disease susceptibility, notably with the ageing process and increased risk of tumour (Hanahan and Weinberg 2011) and neurological disease (Girirajan *et al.* 2011). The size, location and the CNV type (duplication or deletion), as well as the genes located within the CNV, are all factors that contribute to the arising phenotype. It has been estimated that 14.6% of CNVs are found in exons compared with approximately 1.3% of SNPs (Kloosterman *et al.* 2015), suggesting that CNVs may contribute to disease risk at higher rates than previously thought.

Copy number variations are a normal feature of human placenta (Kasak *et al.* 2015; Coorens *et al.* 2021) which increase in number across gestation and also exceed numbers expected in human blood. Numerous studies have added to the evidence for CNV involvement in human pregnancy loss across gestation (Rajcan-Separovic *et al.* 2010*a*, 2010*b*; Levy *et al.* 2014; Nagirnaja *et al.* 2014; Karim *et al.* 2017; Kasak *et al.* 2017; Li *et al.* 2018; Fan *et al.* 2020; Wang *et al.* 2020) and unexplained stillbirth (Harris *et al.* 2011; Ernst *et al.* 2015). A single study to date has profiled the CNVs in normal human placental development (Kasak *et al.* 2015). A three-fold increase of N_{CNV} in placentae was noted compared with the parental blood, and a significant increase in N_{CNV} was found through healthy placental development. Using trios (maternal–paternal–placental), Kasak *et al.* (2015) found 11.1% of the CNVs in the placental tissue were inherited, and were equally inherited from each parent. Research into CNV influence on both normal pregnancy and pregnancy complications in the horse is lacking, although unpublished data in our laboratory suggests this feature will also be important to equine placental function and pathologies.

Final discussion and concluding comments

The first 2 months of equine pregnancy involve transformation of the simple zygote to a sophisticated foeto-placental unit through a series of spatially and temporally co-ordinated developmental events. Perhaps it is not surprising then that this period is frequently found as the most common for a pregnancy to fail, although foetal lethality is possible right up to parturition. Identifying causes of pregnancy loss throughout gestation, remains an area of intense interest of clinicians and researchers alike. As *in vitro* produced embryos become increasingly popular with breeders (Lazzari *et al.* 2020), excitement reignites around application of IVF in the horse (Felix *et al.* 2022), and the need to meet the challenge of managing the negative impact of deleterious mutations in breeds becomes more urgent (Orlando and Librado 2019), it seems no better time to expand our understanding of lethal genetic variants of the equine foetus.

In this review, we identified three types of variants directly associated with pregnancy loss in the mare: aneuploidy (Shilton *et al.* 2020), SNPs (Ducro *et al.* 2015; Monthoux *et al.* 2015) and translocations (Bugno-Poniewierska and Raudsepp 2021), with varying levels of evidence for lethality. Structural variants and SNPs of the mare's genome have been found to increase the risk of that individual suffering an abortion or recurrent early embryonic loss. Presumably these variants act on the environment that mare provides for the developing foetus or lead to an imbalanced gamete

genome which is inherited by the embryo through the germline. Duos have not been assessed to confirm either of these assumptions.

Variants intrinsic to the foeto-placental unit (aneuploidy, SNPs) have been identified that are thought to directly impact the development of the foetus and/or placenta through either (1) modulating the expression and/or function of an individual protein (Monthoux *et al.* 2015) or (2) leading to widespread genome instability (Shilton *et al.* 2020). These foetal variants have stronger evidence for lethality. The National Institute of Child Health and Human Development classifies chromosomal abnormalities as meeting the criteria for causality (Reddy *et al.* 2009). The evidence that equine Trisomy 1, 3, 15, 20, 23/34 and monosomy 27 and 31 are foetal lethal lies the fact that none of these variants have ever been reported in viable horses, despite both large scale and targeted screening by cytogenetic laboratories globally. Using this argument, trisomy 30 in EPL tissue may not be causal for pregnancy loss as it is described in a number of liveborn horses, although heterogenous phenotypes associated with human Trisomy 21 suggests this remains plausible (Hwang *et al.* 2021). It is curious that to date neither confined placental mosaicism nor foetal–placental mosaicism is yet to be identified, but we must not be complacent and assume it does not exist. It may well not be until blastocyst screening takes off that we really will know the true extent, plausible now with expanding numbers of IVP embryos (Lazzari *et al.* 2020; Felix *et al.* 2022). Evidence available up to this point suggests equine aneuploidy likely originates in maternal meiosis, responsible for 84% of human aneuploid conceptuses (Mikwar *et al.* 2020). Based on the criteria of Reddy *et al.* 2009, SNPs in *PLOD1* and *B3GALNT2* also have strong evidence for causality with few or no examples of viable offspring, and clear biological plausibility linking the affected protein and resultant foetal pathology.

In conclusion, research in this field in the horse is very much in its infancy but shows great promise for future diagnostic tests and to inform breeding decisions that avoid lethality. Research on techniques such as non-invasive prenatal testing through the detection of foetally derived DNA in pregnant mare serum has been initiated (Tonekaboni *et al.* 2020; Kadivar *et al.* 2021) and based on its success in women (Guy *et al.* 2021), is likely to be valuable to make this research relevant to clinical practice. To date, there are no foetal only or placental only variants, although it is reasonable to assume with further research these will be revealed in due course.

References

Alberts B, Bray D, Hopkin K, Johnson AD, Lewis J, Raff M, Roberts K, Walter P (2015) 'Essential cell biology.' (Garland Science)

Allen WR (2001) Luteal deficiency and embryo mortality in the mare. *Reproduction in Domestic Animals* **36**(3–4), 121–131. doi:10.1046/j.1439-0531.2001.d01-43.x

Allen WR, Wilsher S (2009) A review of implantation and early placentation in the mare. *Placenta* **30**(12), 1005–1015. doi:10.1016/j.placenta.2009.09.007

Allen WR, Wilsher S (2012) The influence of mare numbers, ejaculation frequency and month on the fertility of Thoroughbred stallions. *Equine Veterinary Journal* **44**(5), 535–541. doi:10.1111/j.2042-3306.2011.00525.x

Allen WR, Brown L, Wright M, Wilsher S (2007) Reproductive efficiency of Flatrace and National Hunt Thoroughbred mares and stallions in England. *Equine Veterinary Journal* **39**(5), 438–445. doi:10.2746/042516407X1737581

Antczak DF, de Mestre AM, Wilsher S, Allen WR (2013) The equine endometrial cup reaction: a fetomaternal signal of significance. *Annual Review of Animal Biosciences* **1**, 419–442. doi:10.1146/annurev-animal-031412-103703

Aurich C, Müller-Herbst S, Reineking W, Müller E, Wohlsein P, Gunreben B, Aurich J (2019) Characterization of abortion, stillbirth and non-viable foals homozygous for the Warmblood Fragile Foal Syndrome. *Animal Reproduction Science* **211**, 106202. doi:10.1016/j.anireprosci.2019.106202

Bain AM (1969) Foetal losses during pregnancy in the thoroughbred mare: a record of 2,562 pregnancies. *New Zealand Veterinary Journal* **17**, 155–158. doi:10.1080/00480169.1969.33811

Ball BA, Hillman RB, Woods GL (1987) Survival of equine embryos transferred to normal and subfertile mares. *Theriogenology* **28**(2), 167–174. doi:10.1016/0093-691X(87)90264-0

Ball BA, Little TV, Weber JA, Woods GL (1989) Survival of Day-4 embryos from young, normal mares and aged, subfertile mares after transfer to normal recipient mares. *Reproduction* **85**(1), 187–194. doi:10.1530/jrf.0.0850187

Barreto RdSN, Rodrigues MN, Carvalho RC, De Oliveira E. Silva FM, Rigoglio NN, Jacob JCF, Gastal EL, Miglino MA (2016) Organogenesis of the musculoskeletal system in horse embryos and early fetuses. *The Anatomical Record* **299**(6), 722–729. doi:10.1002/ar.23339

Bastos HBA, Martinez MN, Camozzato GC, Estradé MJ, Barros E, Vital CE, Vidigal PMP, Meikle A, Jobim MIM, Gregory RM, Mattos RC (2019) Proteomic profile of histotroph during early embryo development in mares. *Theriogenology* **125**, 224–235. doi:10.1016/j.theriogenology.2018.11.002

Battaglia DE, Goodwin P, Klein NA, Soules MR (1996) Fertilization and early embryology: influence of maternal age on meiotic spindle assembly oocytes from naturally cycling women. *Human Reproduction* **11**(10), 2217–2222. doi:10.1093/oxfordjournals.humrep.a019080

Bellone RR, Ocampo NR, Hughes SS, Le V, Arthur R, Finno CJ, Penedo MCT (2020) Warmblood fragile foal syndrome type 1 mutation (PLOD1 c.2032*G*>A) is not associated with catastrophic breakdown and has a low allele frequency in the Thoroughbred breed. *Equine Veterinary Journal* **52**(3), 411–414. doi:10.1111/evj.13182

Betteridge KJ, Raeside JI, Waelchli RO, Christie HL, Hayes MA (2018) Patterns of conceptus development and of progesterone concentrations in maternal blood preceding spontaneous early pregnancy failure in mares. *Reproduction, Fertility and Development* **30**, 1066–1076. doi:10.1071/RD17336

Beyer T, Rink BE, Scarlet D, Walter I, Kunert S, Aurich C (2019) Early luteal phase progestin concentration influences endometrial function in pregnant mares. *Theriogenology* **125**, 236–241. doi:10.1016/j.theriogenology.2018.11.018

Blue MG (1981) A cytogenetical study of prenatal loss in the mare. *Theriogenology* **15**, 295–309. doi:10.1016/0093-691X(81)90051-0

Bosh KA, Powell D, Neibergs JS, Shelton B, Zent W (2009*a*) Impact of reproductive efficiency over time and mare financial value on economic returns among Thoroughbred mares in central Kentucky. *Equine Veterinary Journal* **41**, 889–894. doi:10.2746/042516409X456059

Bosh KA, Powell D, Shelton B, Zent W (2009*b*) Reproductive performance measures among Thoroughbred mares in central Kentucky, during the 2004 mating season. *Equine Veterinary Journal* **41**(9), 883–888. doi:10.2746/042516409X456068

Bowling AT, Millon LV (1990) Two autosomal trisomies in the horse: 64, XX, −26, +t(26q26q) and 65,XX, +30. *Genome* **33**(5), 679–682. doi:10.1139/g90-101

Brito LFC, Sertich PL, Durkin K, Chowdhary BP, Turner RM, Greene LM, McDonnell S (2008) Autosomic 27 trisomy in a Standardbred colt.

Journal of Equine Veterinary Science **28**(7), 431–436. doi:10.1016/j.jevs.2008.06.003

Bugno M, Slota E, Koscielny M (2007) Karyotype evaluation among young horse populations in Poland. *Schweizer Archiv für Tierheilkunde* **149**(5), 227–232. doi:10.1024/0036-7281.149.5.227

Bugno-Poniewierska M, Raudsepp T (2021) Horse clinical cytogenetics: recurrent themes and novel findings. *Animals* **11**(3), 831. doi:10.3390/ani11030831

Bugno-Poniewierska M, Kozub D, Pawlina K, Tischner M Jr, Tischner M, Słota E, Wnuk M (2011) Determination of the correlation between stallion's age and number of sex chromosome aberrations in spermatozoa. *Reproduction in Domestic Animals* **46**(5), 787–792. doi:10.1111/j.1439-0531.2010.01742.x

Bugno-Poniewierska M, Wojtaszek M, Pawlina-Tyszko K, Kowalska K, Witarski W, Raudsepp T (2018) Evaluation of the prevalence of sex chromosome aberrations in a population of young horses—Preliminary results. In 'Proceedings of the Dorothy Russell Havemeyer 12th International Horse Genome Workshop, Pavia, Italy.' pp. 12–15.

Buoen LC, Zhang TQ, Weber AF, Turner T, Bellamy J, Ruth GR (1997) Arthrogryposis in the foal and its possible relation to autosomal trisomy. *Equine Veterinary Journal* **29**(1), 60–62. doi:10.1111/j.2042-3306.1997.tb01638.x

Byrne J, Warburton D, Kline J, Blanc W, Stein Z (1985) Morphology of early fetal deaths and their chromosomal characteristics. *Teratology* **32**, 297–315. doi:10.1002/tera.1420320218

Byrne J, Warburton D, Opitz JM, Reynolds JF (1986) Neural tube defects in spontaneous abortions. *American Journal of Medical Genetics* **25**, 327–333. doi:10.1002/ajmg.1320250219

Chavatte-Palmer P, Derisoud E, Robles M (2022) Pregnancy and placental development in horses: an update. *Domestic Animal Endocrinology* **79**, 106692. doi:10.1016/j.domaniend.2021.106692

Chen S, Liu D, Zhang J, Li S, Zhang L, Fan J, Luo Y, Qian Y, Huang H, Liu C, Zhu H, Jiang Z, Xu C (2017) A copy number variation genotyping method for aneuploidy detection in spontaneous abortion specimens. *Prenatal Diagnosis* **37**, 176–183. doi:10.1002/pd.4986

Chen L, Wang L, Tang F, Zeng Y, Yin D, Zhou C, Zhu H, Li L, Zhang L, Wang J (2021) Copy number variation sequencing combined with quantitative fluorescence polymerase chain reaction in clinical application of pregnancy loss. *Journal of Assisted Reproduction and Genetics* **38**(9), 2397–2404. doi:10.1007/s10815-021-02243-9

Chevalier F, Palmer E (1982) Ultrasonic echography in the mare. *Journal of Reproduction and Fertility. Supplement* **32**, 423–430.

Claes A, Cuervo-Arango J, van den Broek J, Galli C, Colleoni S, Lazzari G, Deelen C, Beitsma M, Stout TA (2019) Factors affecting the likelihood of pregnancy and embryonic loss after transfer of cryopreserved *in vitro* produced equine embryos. *Equine Veterinary Journal* **51**(4), 446–450. doi:10.1111/evj.13028

Conley AJ, Ball BA (2019) Steroids in the establishment and maintenance of pregnancy and at parturition in the mare. *Reproduction* **158**(6), R197–R208. doi:10.1530/REP-19-0179

Coorens THH, Oliver TRW, Sanghvi R, Sovio U, Cook E, Vento-Tormo R, Haniffa M, Young MD, Rahbari R, Sebire N, Campbell PJ, Charnock-Jones DS, Smith GCS, Behjati S (2021) Inherent mosaicism and extensive mutation of human placentas. *Nature* **592**(7852), 80–85 [Erratum in: Nature 2022; 603(7901):E17]. doi:10.1038/s41586-021-03345-1

Crabtree JR, Chang Y, de Mestre AM (2012) Clinical presentation, treatment and possible causes of persistent endometrial cups illustrated by two cases. *Equine Veterinary Education* **24**(5), 251–259. doi:10.1111/j.2042-3292.2011.00354.x

Cuervo-Arango J, Claes AN, Stout TA (2019) A retrospective comparison of the efficiency of different assisted reproductive techniques in the horse, emphasizing the impact of maternal age. *Theriogenology* **132**, 36–44. doi:10.1016/j.theriogenology.2019.04.010

D'Fonseca NMM, Gibson CME, Hummel I, van Doorn DA, Roelfsema E, Stout TAE, van den Broek J, de Ruijter-Villani M (2021) Overfeeding extends the period of annual cyclicity but increases the risk of early embryonic death in Shetland pony mares. *Animals* **11**(2), 361. doi:10.3390/ani11020361

de Leon PMM, Campos VF, Thurow HS, Hartwig FP, Selau LP, Dellagostin OA, Neto JB, Deschamps JC, Seixas FK, Collares T (2012) Association between single nucleotide polymorphisms in p53 and abortion in Thoroughbred mares. *The Veterinary Journal* **193**(2), 573–575. doi:10.1016/j.tvjl.2012.02.003

de Mestre AM, Rose BV, Chang YM, Wathes DC, Verheyen KLP (2019) Multivariable analysis to determine risk factors associated with early pregnancy loss in Thoroughbred broodmares. *Theriogenology* **124**, 18–23. doi:10.1016/j.theriogenology.2018.10.008

Derisoud E, Auclair-Ronzaud J, Palmer E, Robles M, Chavatte-Palmer P (2021) Female age and parity in horses: how and why does it matter? *Reproduction, Fertility and Development* **34**(2), 52–116. doi:10.1071/RD21267

Dias NM, de Andrade DGA, Teixeira-Neto AR, Trinque CM, de Oliveira-Filho JP, Winand NJ, Araújo JP Jr, Borges AS (2019) Warmblood Fragile Foal Syndrome causative single nucleotide polymorphism frequency in Warmblood horses in Brazil. *The Veterinary Journal* **248**, 101–102. doi:10.1016/j.tvjl.2019.05.002

Dijkstra A, Cuervo-Arango J, Stout TAE, Claes A (2020) Monozygotic multiple pregnancies after transfer of single *in vitro* produced equine embryos. *Equine Veterinary Journal* **52**(2), 258–261. doi:10.1111/evj.13146

Ducro BJ, Schurink A, Bastiaansen JWM, Boegheim IJM, van Steenbeek FG, Vos-Loohuis M, Nijman IJ, Monroe GR, Hellinga I, Dibbits BW, Back W, Leegwater PAJ (2015) A nonsense mutation in *B3GALNT2* is concordant with hydrocephalus in Friesian horses. *BMC Genomics* **16**(1), 761. doi:10.1186/s12864-015-1936-z

Durkin K, Raudsepp T, Chowdhary BP (2011) Cytogenetic evaluation of the stallion. In 'Equine reproduction'. (Eds AO McKinnon, WE Vaala, DD Varner) pp. 1462–1468. (Wiley Blackwell)

Ernst LM, Rand CM, Bao R, Andrade J, Linn RL, Minturn L, Zhang C, Kang W, Weese-Mayer DE (2015) Stillbirth: genome-wide copy number variation profiling in archived placental umbilical cord samples with pathologic and clinical correlation. *Placenta* **36**(8), 783–789. doi:10.1016/j.placenta.2015.04.010

Fan L, Wu J, Wu Y, Shi X, Xin X, Li S, Zeng W, Deng D, Feng L, Chen S, Xiao J (2020) Analysis of chromosomal copy number in first-trimester pregnancy loss using next-generation sequencing. *Frontiers in Genetics* **11**, 545856. doi:10.3389/fgene.2020.545856

Fawcett JA, Innan H, Tsuchiya T, Sato F (2021) Effect of advancing age on the reproductive performance of Japanese Thoroughbred broodmares. *Journal of Equine Science* **32**(2), 31–37. doi:10.1294/jes.32.31

Felix MR, Turner RM, Dobbie T, Hinrichs K (2022) Successful *in vitro* fertilization in the horse: production of blastocysts and birth of foals after prolonged sperm incubation for capacitation. *Biology of Reproduction*, ioac172. doi:10.1093/biolre/ioac172

Ferris RA, Sonnis J, Webb B, Lindholm A, Hassel D (2011) Hydrocephalus in an American Miniature Horse foal: a case report and review. *Journal of Equine Veterinary Science* **31**(11), 611–614. doi:10.1016/j.jevs.2011.03.005

Flood PF, Betteridge KJ, Diocee MS (1982) Transmission electron microscopy of horse embryos 3-16 days after ovulation. *Journal of Reproduction and Fertility. Supplement* **32**, 319–327.

Fowden AL, Giussani DA, Forhead AJ (2020) Physiological development of the equine fetus during late gestation. *Equine Veterinary Journal* **52**(2), 165–173. doi:10.1111/evj.13206

Franciolli ALR, Cordeiro BM, da Fonseca ET, Rodrigues MN, Sarmento CAP, Ambrosio CE, de Carvalho AF, Miglino MA, Silva LA (2011) Characteristics of the equine embryo and fetus from days 15 to 107 of pregnancy. *Theriogenology* **76**(5), 819–832. doi:10.1016/j.theriogenology.2011.04.014

Franciosi F, Tessaro I, Dalbies-Tran R, Douet C, Reigner F, Deleuze S, Papillier P, Miclea I, Lodde V, Luciano AM, Goudet G (2017) Analysis of chromosome segregation, histone acetylation, and spindle morphology in horse oocytes. *Journal of Visualized Experiments* (123), e55242. doi:10.3791/55242

Gaivão MMF, Rambags BPB, Stout TAE (2014) Gastrulation and the establishment of the three germ layers in the early horse conceptus. *Theriogenology* **82**(2), 354–365. doi:10.1016/j.theriogenology.2014.04.018

Ghosh S, Qu Z, Das PJ, Fang E, Juras R, Cothran EG, McDonell S, Kenney DG, Lear TL, Adelson DL, Chowdhary BP, Raudsepp T (2014) Copy number variation in the horse genome. *PLoS Genetics* **10**(10), e1004712. doi:10.1371/journal.pgen.1004712

Ghosh S, Das PJ, Avila F, Thwaits BK, Chowdhary BP, Raudsepp T (2016) A non-reciprocal autosomal translocation 64,XX, t(4;10)(q21;p15) in

an Arabian mare with repeated early embryonic loss. *Reproduction in Domestic Animals* **51**(1), 171–174. doi:10.1111/rda.12636

Ghosh S, Carden CF, Juras R, Mendoza MN, Jevit MJ, Castaneda C, Phelps O, Dube J, Kelley DE, Varner DD, Love CC, Raudsepp T (2020) Two novel cases of autosomal translocations in the horse: Warmblood family segregating t(4;30) and a cloned Arabian with a *de novo* t(12;25). *Cytogenetic and Genome Research* **160**(11–12), 688–697. doi:10.1159/000512206

Giles RC, Donahue JM, Hong CB, Tuttle PA, Petrites-Murphy MB, Poonacha KB, Roberts AW, Tramontin RR, Smith B, Swerczek TW (1993) Causes of abortion, stillbirth, and perinatal death in horses: 3,527 cases (1986–1991). *Journal of the American Veterinary Medical Association* **203**(8), 1170–1175.

Ginther OJ (1985) Embryonic loss in mares: incidence, time of occurrence, and hormonal involvement. *Theriogenology* **23**(1), 77–89. doi:10.1016/0093-691X(85)90074-3

Ginther OJ (1998) Equine pregnancy: physical interactions between the uterus and conceptus. In 'Proceedings of the American Association of Equine Practitioners'. pp. 73–104.

Ginther OJ, Griffin PG (1994) Natural outcome and ultrasonic identification of equine fetal twins. *Theriogenology* **41**, 1193–1199. doi:10.1016/S0093-691X(05)80041-X

Girirajan S, Brkanac Z, Coe BP, Baker C, Vives L, Vu TH, Shafer N, Bernier R, Ferrero GB, Silengo M, Warren ST, Moreno CS, Fichera M, Romano C, Raskind WH, Eichler EE (2011) Relative burden of large CNVs on a range of neurodevelopmental phenotypes. *PLoS Genetics* **7**(11), e1002334. doi:10.1371/journal.pgen.1002334

Govaere J, Hoogewijs M, De Schauwer C, Van Zeveren A, Smits K, Cornillie P, de Kruif A (2009) An abortion of monozygotic twins in a warmblood mare. *Reproduction in Domestic Animals = Zuchthygiene* **44**, 852–854. doi:10.1111/j.1439-0531.2008.01112.x

Grillos AS, Roach JM, de Mestre AM, Foote AK, Kinglsey NB, Mienaltowski MJ, Bellone RR (2022) First reported case of fragile foal syndrome type 1 in the Thoroughbred caused by PLOD1 c.2032G>A. *Equine Veterinary Journal* **54**(6), 1086–1093. doi:10.1111/evj.13547

Grüninger B, Schoon H-A, Schoon D, Menger S, Klug E (1998) Incidence and morphology of endometrial angiopathies in mares in relationship to age and parity. *Journal of Comparative Pathology* **119**(3), 293–309. doi:10.1016/S0021-9975(98)80051-0

Guy GP, Hargrave J, Dunn R, Price K, Short J, Thilaganathan B, on behalf of the SAFE test collaborative (2021) Secondary non-invasive prenatal screening for fetal trisomy: an effectiveness study in a public health setting. *BJOG: An International Journal of Obstetrics & Gynaecology* **128**, 440–446. doi:10.1111/1471-0528.16464

Hamstead L, Chang Y-M, Crowhurst J, Wise Z, McGladdery A, Ricketts S, de Mestre AM (2012) Retrospective study of early pregnancy loss in Thoroughbred mares. *Equine Veterinary Journal* **44**(s42), 5.

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* **144**(5), 646–674. doi:10.1016/j.cell.2011.02.013

Hanlon DW, Stevenson M, Evans MJ, Firth EC (2012) Reproductive performance of Thoroughbred mares in the Waikato region of New Zealand: 2. Multivariable analyses and sources of variation at the mare, stallion and stud farm level. *New Zealand Veterinary Journal* **60**(6), 335–343. doi:10.1080/00480169.2012.696240

Harris RA, Ferrari F, Ben-Shachar S, Wang X, Saade G, Van Den Veyver I, Facchinetti F, Aagaard-Tillery K (2011) Genome-wide array-based copy number profiling in human placentas from unexplained stillbirths. *Prenatal Diagnosis* **31**(10), 932–944. doi:10.1002/pd.2817

Hassold T, Hall H, Hunt P (2007) The origin of human aneuploidy: where we have been, where we are going. *Human Molecular Genetics* **16**(R2), R203–R208. doi:10.1093/hmg/ddm243

Haynes SE, Reisner AH (1982) Cytogenetic and DNA analyses of equine abortion. *Cytogenetic and Genome Research* **34**, 204–214. doi:10.1159/000131808

Hemberg E, Lundeheim N, Einarsson S (2004) Reproductive performance of Thoroughbred mares in Sweden. *Reproduction in Domestic Animals* **39**, 81–85. doi:10.1111/j.1439-0531.2004.00482.x

Holl HM, Lear TL, Nolen-Walston RD, Slack J, Brooks SA (2013) Detection of two equine trisomies using SNP-CGH. *Mammalian Genome* **24**(5), 252–256. doi:10.1007/s00335-013-9450-6

Hollinshead FK, Mehegan MK, Gunn A, Nett T, Bruemmer JE, Hanlon DW (2022) The correlation of endogenous progesterone concentration in diestrus on early pregnancy rate in Thoroughbred mares. *Journal of Equine Veterinary Science* **118**, 104127. doi:10.1016/j.jevs.2022.104127

Hong CB, Donahue JM, Giles RC Jr, Petrites-Murphy MB, Poonacha KB, Roberts AW, Smith BJ, Tramontin RR, Tuttle PA, Swerczek TW (1993) Equine abortion and stillbirth in Central Kentucky during 1988 and 1989 foaling seasons. *Journal of Veterinary Diagnostic Investigation* **5**(4), 560–566. doi:10.1177/104063879300500410

Hornak M, Oracova E, Hulinska P, Urbankova L, Rubes J (2012) Aneuploidy detection in pigs using comparative genomic hybridization: from the oocytes to blastocysts. *PLoS ONE* **7**, e30335. doi:10.1371/journal.pone.0030335

Hwang S, Cavaliere P, Li R, Zhu LJ, Dephoure N, Torres EM (2021) Consequences of aneuploidy in human fibroblasts with trisomy 21. *Proceedings of the National Academy of Sciences of the United States of America* **118**(6), e2014723118. doi:10.1073/pnas.2014723118

Irvine CH, Sutton P, Turner JE, Mennick PE (1990) Changes in plasma progesterone concentrations from Days 17 to 42 of gestation in mares maintaining or losing pregnancy. *Equine Veterinary Journal* **22**(2), 104–106. doi:10.1111/j.2042-3306.1990.tb04219.x

Irwin CF (1975) Early pregnancy testing and its relationship to abortion. *Journal of Reproduction and Fertility. Supplement* 485–488.

Jeffcott LB, Whitwell KE (1973) Twinning as a cause of foetal and neonatal loss in the Thoroughbred mare. *Journal of Comparative Pathology* **83**, 91–106. doi:10.1016/0021-9975(73)90032-7

Jia C-W, Wang L, Lan Y-L, Song R, Zhou L-Y, Yu L, Yang Y, Liang Y, Li Y, Ma Y-M, Wang S-Y (2015) Aneuploidy in early miscarriage and its related factors. *Chinese Medical Journal* **128**, 2772–2776. doi:10.4103/0366-6999.167352

Kadivar A, Rashidzadeh H, Davoodian N, Nazari H, Dehghani Tafti R, Heidari Khoei H, Seidi Samani H, Modaresi J, Ahmadi E (2021) Evaluation of the efficiency of TaqMan duplex real-time PCR assay for noninvasive prenatal assessment of fetal sex in equine. *Reproduction in Domestic Animals* **56**(2), 287–291. doi:10.1111/rda.13831

Kahler A, McGonnell IM, Smart H, Kowalski AA, Smith KC, Wathes DC, de Mestre AM (2021) Fetal morphological features and abnormalities associated with equine early pregnancy loss. *Equine Veterinary Journal* **53**, 530–541. doi:10.1111/evj.13340

Karim S, Jamal HS, Rouzi A, Ardawi MSM, Schulten H-J, Mirza Z, Alansari NA, Al-Quaiti MM, Abusamra H, Naseer MI, Turki R, Chaudhary AG, Gari M, Abuzenadah AM, Al-Qhatani MH (2017) Genomic answers for recurrent spontaneous abortion in Saudi Arabia: an array comparative genomic hybridization approach. *Reproductive Biology* **17**(2), 133–143. doi:10.1016/j.repbio.2017.03.003

Kasak L, Rull K, Vaas P, Teesalu P, Laan M (2015) Extensive load of somatic CNVs in the human placenta. *Scientific Reports* **5**(1), 8342. doi:10.1038/srep08342

Kasak L, Rull K, Sõber S, Laan M (2017) Copy number variation profile in the placental and parental genomes of recurrent pregnancy loss families. *Scientific Reports* **7**(1), 45327. doi:10.1038/srep45327

Kasarskis A, Manova K, Anderson KV (1998) A phenotype-based screen for embryonic lethal mutations in the mouse. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 7485–7490. doi:10.1073/pnas.95.13.7485

Katila T, Ferreira-Dias G (2022) Evolution of the concepts of endometrosis, post breeding endometritis, and susceptibility of mares. *Animals* **12**(6), 779. doi:10.3390/ani12060779

Kloosterman WP, Francioli LC, Hormozdiari F, Marschall T, Hehir-Kwa JY, Abdellaoui A, Lameijer E-W, Moed MH, Koval V, Renkens I, van Roosmalen MJ, Arp P, Karssen LC, Coe BP, Handsaker RE, Suchiman ED, Cuppen E, Thung DT, McVey M, Wendl MC, van Duijn CM, Swertz MA, Wijmenga C, van Ommen GJB, Slagboom PE, Boomsma DI, Schönhuth A, Eichler EE, de Bakker PIW, Ye K, Guryev V (2015) Characteristics of *de novo* structural changes in the human genome. *Genome Research* **25**(6), 792–801. doi:10.1101/gr.185041.114

Klunder LR, McFeely RA, Beech J, McClune W, Bilinski WF (1989) Autosomal trisomy in a Standardbred colt. *Equine Veterinary Journal* **21**(1), 69–70. doi:10.1111/j.2042-3306.1989.tb02092.x

Köhne M, Kuhl J, Ille N, Erber R, Aurich C (2014) Treatment with human chorionic gonadotrophin before ovulation increases progestin concentration in early equine pregnancies. *Animal Reproduction Science* **149**(3–4), 187–193. doi:10.1016/j.anireprosci.2014.07.002

Kolb DS, Klein C (2019) Congenital hydrocephalus in a Belgian draft horse associated with a nonsense mutation in B3GALNT2. *The Canadian Veterinary Journal* **60**, 197–198.

Kubien EM, Tischner M (2002) Reproductive success of a mare with a mosaic karyotype: 64,XX/65,XX,+30. *Equine Veterinary Journal* **34**, 99–100. doi:10.2746/042516402776181240

Lane EA, Bijnen MLJ, Osborne M, More SJ, Henderson ISF, Duffy P, Crowe MA (2016) Key factors affecting reproductive success of Thoroughbred mares and stallions on a commercial stud farm. *Reproduction in Domestic Animals* **51**(2), 181–187. doi:10.1111/rda.12655

Lazzari G, Colleoni S, Crotti G, Turini P, Fiorini G, Barandalla M, Landriscina L, Dolci G, Benedetti M, Duchi R, Galli C (2020) Laboratory production of equine embryos. *Journal of Equine Veterinary Science* **89**, 103097. doi:10.1016/j.jevs.2020.103097

Lear TL, Layton G (2002) Use of Zoo-FISH to characterise a reciprocal translocation in a Thoroughbred mare: t(1;16)(q16;q21.3). *Equine Veterinary Journal* **34**, 207–209. doi:10.2746/042516402776767295

Lear TL, Cox JH, Kennedy GA (1999) Autosomal trisomy in a Thoroughbred colt: 65,XY,+31. *Equine Veterinary Journal* **31**(1), 85–88. doi:10.1111/j.2042-3306.1999.tb03796.x

Lear TL, Lundquist J, Zent WW, Fishback WD Jr, Clark A (2008) Three autosomal chromosome translocations associated with repeated early embryonic loss (REEL) in the domestic horse (*Equus caballus*). *Cytogenetic and Genome Research* **120**, 117–122. doi:10.1159/000118749

Lear TL, Raudsepp T, Lundquist JM, Brown SE (2014) Repeated early embryonic loss in a Thoroughbred mare with a chromosomal translocation [64,XX,t(2;13)]. *Journal of Equine Veterinary Science* **34**, 805–809. doi:10.1016/j.jevs.2014.01.007

Levy B, Sigurjonsson S, Pettersen B, Maisenbacher MK, Hall MP, Demko Z, Lathi RB, Tao R, Aggarwal V, Rabinowitz M (2014) Genomic imbalance in products of conception: single-nucleotide polymorphism chromosomal microarray analysis. *Obstetrics & Gynecology* **124**(2 PART 1), 202–209. doi:10.1097/AOG.0000000000000325

Li H, Liu M, Xie M, Zhang Q, Xiang J, Duan C, Ding Y, Liu Y, Mao J, Wang T, Li H (2018) Submicroscopic chromosomal imbalances contribute to early abortion. *Molecular Cytogenetics* **11**(1), 41. doi:10.1186/s13039-018-0386-0

Long SE (1996) Tandem 1;30 translocation: a new structural abnormality in the horse (*Equus caballus*). *Cytogenetic and Genome Research* **72**, 162–163. doi:10.1159/000134176

Macdonald AA, Chavatte P, Fowden AL (2000) Scanning electron microscopy of the microcotyledonary placenta of the horse (*Equus caballus*) in the latter half of gestation. *Placenta* **21**(5–6), 565–574. doi:10.1053/plac.2000.0510

Macleay CM, Carrick J, Shearer P, Begg A, Stewart M, Heller J, Chicken C, Brookes VJ (2022) A scoping review of the global distribution of causes and syndromes associated with mid- to late-term pregnancy loss in horses between 1960 and 2020. *Veterinary Sciences* **9**(4), 186. doi:10.3390/vetsci9040186

Martin RH (2007) The clinical relevance of sperm aneuploidy. In 'The genetics of male infertility'. (Ed. DT Carrell) pp. 129–144. (Humana Press)

Meadows SJ, Binns MM, Newcombe JR, Thompson CJ, Rossdale PD (1995) Identical triplets in a Thoroughbred mare. *Equine Veterinary Journal* **27**, 394–397. doi:10.1111/j.2042-3306.1995.tb04076.x

Mendoza MN, Schalnus SA, Thomson B, Bellone RR, Juras R, Raudsepp T (2020) Novel complex unbalanced dicentric X-autosome rearrangement in a Thoroughbred mare with a mild effect on the phenotype. *Cytogenetic and Genome Research* **160**, 597–609. doi:10.1159/000511236

Mikwar M, MacFarlane AJ, Marchetti F (2020) Mechanisms of oocyte aneuploidy associated with advanced maternal age. *Mutation Research/Reviews in Mutation Research* **785**, 108320. doi:10.1016/j.mrrev.2020.108320

Miyakoshi D, Shikichi M, Ito K, Iwata K, Okai K, Sato F, Nambo Y (2012) Factors influencing the frequency of pregnancy loss among Thoroughbred mares in Hidaka, Japan. *Journal of Equine Veterinary Science* **32**(9), 552–557. doi:10.1016/j.jevs.2012.01.003

Monthoux C, de Brot S, Jackson M, Bleul U, Walter J (2015) Skin malformations in a neonatal foal tested homozygous positive for Warmblood Fragile Foal Syndrome. *BMC Veterinary Research* **11**(1), 12. doi:10.1186/s12917-015-0318-8

Morehead JP, Blanchard TL, Thompson JA, Brinsko SP (2002) Evaluation of early fetal losses on four equine farms in central Kentucky: 73 cases (2001). *Journal of the American Veterinary Medical Association* **220**(12), 1828–1830. doi:10.2460/javma.2002.220.1828

Morley PS, Townsend HGG (1997) A survey of reproductive performance in Thoroughbred mares and morbidity, mortality and athletic potential of their foals. *Equine Veterinary Journal* **29**, 290–297. doi:10.1111/j.2042-3306.1997.tb03126.x

Morris LH, Allen WR (2002) Reproductive efficiency of intensively managed Thoroughbred mares in Newmarket. *Equine Veterinary Journal* **34**, 51–60. doi:10.2746/042516402776181222

Morris LHA, McCue PM, Aurich C (2020) Equine endometritis: a review of challenges and new approaches. *Reproduction* **160**, R95–R110. doi:10.1530/REP-19-0478

Mouncey R, Arango-Sabogal JC, de Mestre AM, Foote AK, Verheyen KL (2022) Retrospective analysis of post-mortem findings in Thoroughbreds aged from birth to 18 months presented to a UK pathology laboratory. *The Veterinary Journal* **281**, 105813. doi:10.1016/j.tvjl.2022.105813

Nagirnaja L, Palta P, Kasak L, Rull K, Christiansen OB, Nielsen HS, Steffensen R, Esko T, Remm M, Laan M (2014) Structural genomic variation as risk factor for idiopathic recurrent miscarriage. *Human Mutation* **35**(8), 972–982. doi:10.1002/humu.22589

Nath LC, Anderson GA, McKinnon AO (2010) Reproductive efficiency of Thoroughbred and Standardbred horses in north-east Victoria. *Australian Veterinary Journal* **88**(5), 169–175. doi:10.1111/j.1751-0813.2010.00565.x

Nieto-Olmedo P, Martín-Cano FE, Gaitskell-Phillips G, Ortiz-Rodríguez JM, Peña FJ, Ortega-Ferrusola C (2020) Power Doppler can detect the presence of 7–8 day conceptuses prior to flushing in an equine embryo transfer program. *Theriogenology* **145**, 1–9. doi:10.1016/j.theriogenology.2020.01.015

Ojala M, Ala-Huikku J (1992) Inheritance of hydrocephalus in horses. *Equine Veterinary Journal* **24**(2), 140–143. doi:10.1111/j.2042-3306.1992.tb02799.x

Okada CTC, Kaps M, Perez Quesada J, Gautier C, Aurich J, Aurich C (2020) Diestrous ovulations in pregnant mares as a response to low early postovulatory progestogen concentration. *Animals* **10**(12), 2249. doi:10.3390/ani10122249

Oriol JG, Sharom FJ, Betteridge KJ (1993) Developmentally regulated changes in the glycoproteins of the equine embryonic capsule. *Reproduction* **99**(2), 653–664. doi:10.1530/jrf.0.0990653

Orlando L, Librado P (2019) Origin and evolution of deleterious mutations in horses. *Genes* **10**, 649. doi:10.3390/genes10090649

Outram AK, Stear NA, Bendrey R, Olsen S, Kasparov A, Zaibert V, Thorpe N, Evershed RP (2009) The earliest horse harnessing and milking. *Science* **323**(5919), 1332–1335. doi:10.1126/science.1168594

Pannu AS, Singh P (2014) Dystocia due to fetal hydrocephalus and its management in a mare. *Intas Polivet* **15**(2), 363–364.

Power MM (1987) Equine half sibs with an unbalanced X;15 translocation or trisomy 28. *Cytogenetic and Genome Research* **45**, 163–168. doi:10.1159/000132448

Power MM (1991) The first description of a balanced reciprocal translocation [t(1q;3q)] and its clinical effects in a mare. *Equine Veterinary Journal* **23**, 146–149. doi:10.1111/j.2042-3306.1991.tb02742.x

Power MM, Gustavsson I, Świtoński M, Plöen L (1992) Synaptonemal complex analysis of an autosomal trisomy in the horse. *Cytogenetic and Genome Research* **61**, 202–207. doi:10.1159/000133408

PriceWaterhouseCoopers LLP (2018) The contribution of thoroughbred breeding to the UK economy and factors impacting the industry's supply chain. Available from: https://www.thetba.co.uk/wp-content/uploads/2018/09/TBA-Economic-Impact-Study-2018.pdf [Accessed 1 August 2022]

Quenby S, Gallos ID, Dhillon-Smith RK, Podesek M, Stephenson MD, Fisher J, Brosens JJ, Brewin J, Ramhorst R, Lucas ES, McCoy RC, Anderson R, Daher S, Regan L, Al-Memar M, Bourne T, MacIntyre DA, Rai R, Christiansen OB, Sugiura-Ogasawara M, Odendaal J, Devall AJ, Bennett PR, Petrou S, Coomarasamy A (2021) Miscarriage matters: the epidemiological, physical, psychological, and economic costs of early pregnancy loss. *The Lancet* **397**(10285), 1658–1667. doi:10.1016/S0140-6736(21)00682-6

Rajcan-Separovic E, Diego-Alvarez D, Robinson WP, Tyson C, Qiao Y, Harvard C, Fawcett C, Kalousek D, Philipp T, Somerville MJ,

Stephenson MD (2010*a*) Identification of copy number variants in miscarriages from couples with idiopathic recurrent pregnancy loss. *Human Reproduction* **25**(11), 2913–2922. doi:10.1093/humrep/deq202

Rajcan-Separovic E, Qiao Y, Tyson C, Harvard C, Fawcett C, Kalousek D, Stephenson M, Philipp T (2010*b*) Genomic changes detected by array CGH in human embryos with developmental defects. *Molecular Human Reproduction* **16**(2), 125–134. doi:10.1093/molehr/gap083

Rambags B, Krijtenburg P, Drie Hv, Lazzari G, Galli C, Pearson P, Colenbrander B, Stout T (2005) Numerical chromosomal abnormalities in equine embryos produced *in vivo* and *in vitro*. *Molecular Reproduction and Development* **72**(1), 77–87. doi:10.1002/mrd.20302

Raudsepp T (2020) Genetics of equine reproductive diseases. *Veterinary Clinics of North America: Equine Practice* **36**(2), 395–409. doi:10.1016/j.cveq.2020.03.013

Raudsepp T, Finno CJ, Bellone RR, Petersen JL (2019) Ten years of the horse reference genome: insights into equine biology, domestication and population dynamics in the post-genome era. *Animal Genetics* **50**(6), 569–597. doi:10.1111/age.12857

Read JE, Cabrera-Sharp V, Offord V, Mirczuk SM, Allen SP, Fowkes RC, de Mestre AM (2018) Dynamic changes in gene expression and signalling during trophoblast development in the horse. *Reproduction* **156**(4), 313–330. doi:10.1530/REP-18-0270

Reddy UM, Goldenberg R, Silver R, Smith GCS, Pauli RM, Wapner RJ, *et al.* (2009) Stillbirth classification—developing an international consensus for research: executive summary of a National Institute of Child Health and Human Development workshop. *Obstetrics & Gynecology* **114**(4), 901–914. doi:10.1097/AOG.0b013e3181b8f6e4

Reiter S, Wallner B, Brem G, Haring E, Hoelzle L, Stefaniuk-Szmukier M, Długosz B, Piórkowska K, Ropka-Molik K, Malvick J, Penedo MCT, Bellone RR (2020) Distribution of the Warmblood Fragile Foal Syndrome type 1 mutation (PLOD1 c.2032G>A) in different horse breeds from Europe and the United States. *Genes* **11**(12), 1518. doi:10.3390/genes11121518

Resende HL, Carmo MT, Ramires Neto C, Alvarenga MA (2014) Determination of equine fetal sex by Doppler ultrasonography of the gonads. *Equine Veterinary Journal* **46**(6), 756–758. doi:10.1111/evj.12213

Ricketts SW, Barrelet A, Whitwell KE (2003) Equine abortion. *Equine Veterinary Education* **15**, 18–21. doi:10.1111/j.2042-3292.2003.tb01809.x

Rizzo M, Ducheyne KD, Deelen C, Beitsma M, Cristarella S, Quartuccio M, Stout TAE, de Ruijter-Villani M (2019) Advanced mare age impairs the ability of *in vitro*-matured oocytes to correctly align chromosomes on the metaphase plate. *Equine Veterinary Journal* **51**(2), 252–257. doi:10.1111/evj.12995

Rizzo M, du Preez N, Ducheyne KD, Deelen C, Beitsma MM, Stout TAE, de Ruijter-Villani M (2020) The horse as a natural model to study reproductive aging-induced aneuploidy and weakened centromeric cohesion in oocytes. *Aging (Albany NY)* **12**, 22220–22232. doi:10.18632/aging.104159

Roach JM, Foote AK, Smith KC, Verheyen KL, de Mestre AM (2021) Incidence and causes of pregnancy loss after Day 70 of gestation in Thoroughbreds. *Equine Veterinary Journal* **53**(5), 996–1003. doi:10.1111/evj.13386

Robles M, Dubois C, Gautier C, Dahirel M, Guenon I, Bouraima-Lelong H, Viguié C, Wimel L, Couturier-Tarrade A, Chavatte-Palmer P (2018) Maternal parity affects placental development, growth and metabolism of foals until 1 year and a half. *Theriogenology* **108**, 321–330. doi:10.1016/j.theriogenology.2017.12.019

Robles M, Loux S, de Mestre AM, Chavatte-Palmer P (2022) Environmental constraints and pathologies that modulate equine placental genes and development. *Reproduction* **163**(3), R25–R38. doi:10.1530/REP-21-0116

Rodrigues MN, Carvalho RC, Franciolli ALR, Rodrigues RF, Rigoglio NN, Jacob JCF, Gastal EL, Miglino MA (2014) Prenatal development of the digestive system in the horse. *The Anatomical Record* **297**(7), 1218–1227. doi:10.1002/ar.22929

Rose BV, Cabrera-Sharp V, Firth MJ, Barrelet FE, Bate S, Cameron IJ, Crabtree JR, Crowhurst J, McGladdery AJ, Neal H, Pynn J, Pynn OD, Smith C, Wise Z, Verheyen KLP, Wathes DC, de Mestre AM (2016) A method for isolating and culturing placental cells from failed early equine pregnancies. *Placenta* **38**, 107–111. doi:10.1016/j.placenta.2015.12.014

Rose BV, Firth M, Morris B, Roach JM, Wathes DC, Verheyen KLP, de Mestre AM (2018) Descriptive study of current therapeutic practices, clinical reproductive findings and incidence of pregnancy loss in intensively managed Thoroughbred mares. *Animal Reproduction Science* **188**, 74–84. doi:10.1016/j.anireprosci.2017.11.011

Ruiz AJ, Castaneda C, Raudsepp T, Tibary A (2019) Azoospermia and Y chromosome–autosome translocation in a Friesian stallion. *Journal of Equine Veterinary Science* **82**, 102781. doi:10.1016/j.jevs.2019.07.002

Schmutz SM, Moker JS, Clark EG, Orr JP (1996) Chromosomal aneuploidy associated with spontaneous abortions and neonatal losses in cattle. *Journal of Veterinary Diagnostic Investigation* **8**, 91–95. doi:10.1177/104063879600800114

Sharma S, Dhaliwal GS, Dadarwal D (2010) Reproductive efficiency of Thoroughbred mares under Indian subtropical conditions: a retrospective survey over 7 years. *Animal Reproduction Science* **117**, 241–248. doi:10.1016/j.anireprosci.2009.05.011

Shilton CA, Kahler A, Davis BW, Crabtree JR, Crowhurst J, McGladdery AJ, Wathes DC, Raudsepp T, de Mestre AM (2020) Whole genome analysis reveals aneuploidies in early pregnancy loss in the horse. *Scientific Reports* **10**(1), 13314. doi:10.1038/s41598-020-69967-z

Simpson DJ, Greenwood RE, Ricketts SW, Rossdale PD, Sanderson M, Allen WR (1982) Use of ultrasound echography for early diagnosis of single and twin pregnancy in the mare. *Journal of Reproduction and Fertility. Supplement* **32**, 431–439.

Sipma KD, Cornillie P, Saulez MN, Stout TAE, Voorhout G, Back W (2013) Phenotypic characteristics of hydrocephalus in stillborn Friesian foals. *Veterinary Pathology* **50**, 1037–1042. doi:10.1177/0300985813488955

Skuse D, Printzlau F, Wolstencroft J (2018) Sex chromosome aneuploidies. In 'Handbook of clinical neurology. Vol. 147'. (Eds DH Geschwind, HL Paulson, C Klein) pp. 355–376. (Elsevier)

Smieszek A, Marcinkowska K, Pielok A, Sikora M, Valihrach L, Carnevale E, Marycz K (2022) Obesity affects the proliferative potential of equine endometrial progenitor cells and modulates their molecular phenotype associated with mitochondrial metabolism. *Cells* **11**(9), 1437. doi:10.3390/cells11091437

Stanton MB, Steiner JV, Pugh DG (2004) Endometrial cysts in the mare. *Journal of Equine Veterinary Science* **24**, 14–19. doi:10.1016/j.jevs.2003.12.003

Stout TA, Allen WR (2002) Prostaglandin E(2) and F(2 alpha) production by equine conceptuses and concentrations in conceptus fluids and uterine flushings recovered from early pregnant and dioestrous mares. *Reproduction* **123**(2), 261–268. doi:10.1530/rep.0.1230261

Stout TAE, Meadows S, Allen WR (2005) Stage-specific formation of the equine blastocyst capsule is instrumental to hatching and to embryonic survival *in vivo*. *Animal Reproduction Science* **87**(3–4), 269–281. doi:10.1016/j.anireprosci.2004.11.009

Su M-T, Lin S-H, Chen Y-C (2011) Genetic association studies of angiogenesis- and vasoconstriction-related genes in women with recurrent pregnancy loss: a systematic review and meta-analysis. *Human Reproduction Update* **17**(6), 803–812. doi:10.1093/humupd/dmr027

Swegen A (2021) Maternal recognition of pregnancy in the mare: does it exist and why do we care? *Reproduction* **161**, R139–R155. doi:10.1530/REP-20-0437

Swegen A, Grupen CG, Gibb Z, Baker MA, de Ruijter-Villani M, Smith ND, Stout TAE, Aitken RJ (2017) From peptide masses to pregnancy maintenance: a comprehensive proteomic analysis of the early equine embryo secretome, blastocoel fluid, and capsule. *Proteomics* **17**(17–18), 1600433. doi:10.1002/pmic.201600433

Todd ET, Thomson PC, Hamilton NA, Ang RA, Lindgren G, Viklund Å, Eriksson S, Mikko S, Strand E, Velie BD (2020) A genome-wide scan for candidate lethal variants in Thoroughbred horses. *Scientific Reports* **10**(1), 13153. doi:10.1038/s41598-020-68946-8

Tonekaboni FR, Narenjisani R, Staji H, Ahmadi-Hamedani M (2020) Comparison of cell-free fetal DNA plasma content used to sex determination between three trimesters of pregnancy in Torkaman pregnant mare. *Journal of Equine Veterinary Science* **95**, 103273. doi:10.1016/j.jevs.2020.103273

Torres EM, Williams BR, Amon A (2008) Aneuploidy: cells losing their balance. *Genetics* **179**(2), 737–746. doi:10.1534/genetics.108.090878

Turner RM (2019) Declining testicular function in the aging stallion: management options and future therapies. *Animal Reproduction Science* **207**, 171–179. doi:10.1016/j.anireprosci.2019.06.009

Ursell PC, Byrne JM, Strobino BA (1985) Significance of cardiac defects in the developing fetus: a study of spontaneous abortuses. *Circulation* **72**, 1232–1236. doi:10.1161/01.CIR.72.6.1232

Villahoz MD, Squires EL, Voss JL, Shideler RK (1985) Some observations on early embryonic death in mares. *Theriogenology* **23**, 915–924. doi:10.1016/0093-691X(85)90009-3

Wang Y, Li Y, Chen Y, Zhou R, Sang Z, Meng L, Tan J, Qiao F, Bao Q, Luo D, Peng C, Wang YS, Luo C, Hu P, Xu Z (2020) Systematic analysis of copy-number variations associated with early pregnancy loss. *Ultrasound in Obstetrics & Gynecology* **55**(1), 96–104. doi:10.1002/uog.20412

West JD, Everett CA (2022) Preimplantation chromosomal mosaics, chimaeras and confined placental mosaicism. *Reproduction and Fertility* **3**(2), R66–R90. doi:10.1530/RAF-21-0095

Willmann C, Schuler G, Hoffmann B, Parvizi N, Aurich C (2011) Effects of age and altrenogest treatment on conceptus development and secretion of LH, progesterone and eCG in early-pregnant mares. *Theriogenology* **75**(3), 421–428. doi:10.1016/j.theriogenology.2010.05.009

Wilsher S, Allen WR (2011) Factors influencing equine chorionic gonadotrophin production in the mare. *Equine Veterinary Journal* **43**(4), 430–438. doi:10.1111/j.2042-3306.2010.00309.x

Wilsher S, Rigali F, Kovacsy S, Allen WRT (2020) Puncture of the equine embryonic capsule and its repair *in vivo* and *in vitro*. *Journal of Equine Veterinary Science* **93**, 103194. doi:10.1016/j.jevs.2020.103194

Yang Y-J, Cho G-J (2007) Factors concerning early embryonic death in Thoroughbred mares in South Korea. *Journal of Veterinary Medical Science* **69**, 787–792. doi:10.1292/jvms.69.787

Zarrei M, MacDonald JR, Merico D, Scherer SW (2015) A copy number variation map of the human genome. *Nature Reviews Genetics* **16**(3), 172–183. doi:10.1038/nrg3871

Zhang T, Bellamy J, Buwn L, Weber A, Ruth G (1992) Autosomal trisomy in a foal with contracted tendon syndrome. In 'Proceedings of the 10th European Colloqium on Cytogenetics of Domestic Animals, Utrecht, The Netherlands'. pp. 18–21.

Data availability. All data is available in the original manuscripts discussed in this review.

Conflicts of interest. The authors declare no conflicts of interest.

Declaration of funding. This research did not receive any specific funding. Authors were funded for related work by Horserace Betting Levy Board and Thoroughbred Breeders Association.

Acknowledgements. The authors would like to thank Anastasia Margaroli.

Author affiliations

[A]Department of Comparative Biomedical Sciences, The Royal Veterinary College, Hawkshead Lane, Herts, AL9 7TA, UK.

[B]Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX 77843-4458, USA.