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Treatment with human chorionic gonadotrophin before ovulation increases progestin concentration in early equine pregnancies

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ABSTRACT

For prevention of early conceptus loss in the horse, treatment with progestins has become common practice. In cattle, treatment with human chorionic gonadotrophin (hCG) during the early postovulatory phase stimulates endogenous progesterone synthesis, which is an important factor for maintenance of early pregnancy via stimulation of endometrial function and conceptus development. In the present study we have therefore investigated the influence of treatment with hCG either for induction of ovulation or during the early luteal phase on plasma progestin concentrations, size of the corpus luteum and size of the conceptus in early pregnant mares. We hypothesized that hCG treatment stimulates progestin secretion and conceptus development. In Experiment 1, induction of ovulation with hCG (1500 IU i.v.; $n = 14$) significantly increased progestin concentration between days 5 and 15 after ovulation compared to untreated controls ($n = 28$; $p < 0.05$; e.g. day 5 hCG i.v.: 17.2 ± 1.9, control: 13.9 ± 0.8 ng/ml). A significant interaction ($p < 0.05$) of hCG treatment with size of the conceptus between days 30 and 40 of pregnancy was detected. In Experiment 2, treatment of mares with hCG (5000 IU) on day 5 after ovulation ($n = 12$) did neither affect progestin secretion (e.g. day 8 hCG: 15.4 ± 1.6, control: 17.6 ± 1.2 ng/ml) nor luteal tissue area (e.g. day 8 hCG: 9.0 ± 0.7, control: 7.6 ± 1.4 cm^2) compared to untreated mares ($n = 9$). In conclusion, treatment of mares with hCG for induction of ovulation within 48 h before ovulation but not on day 5 of the luteal phase stimulates progestin secretion and may enhance conceptus development via stimulation of endometrial function during early pregnancy.

1. Introduction

In horse mares, the risk of conceptus loss during early pregnancy before implantation is higher than at any other phase of gestation (Morris and Allen, 2002). The prevention of conceptus loss during this period is therefore of particular interest. So far, equine early pregnancy loss has been mainly attributed to disturbed or delayed conceptus development (Ginther et al., 1985; Vanderwall et al., 2000), but it is rarely caused by a preceding fall in maternal plasma progesterone concentration (Irvine et al., 1990).

In cattle, progesterone secretion during the early luteal phase contributes to down-regulation of endometrial progesterone receptors (Okumu et al., 2010), which is a

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prerequisite for induction of endometrial receptivity to the conceptus. Via an earlier down-regulation of progesterone receptors high progesterone concentration stimulates conceptus elongation (Clemente et al., 2009) and interferon τ secretion, thus increasing pregnancy rate (Kerbler et al., 1997; Mann et al., 2006; Clemente et al., 2009; Okumu et al., 2010). The importance of progesterone to overcome critical phases of equine conceptus development has been suggested over 20 years ago (Kastelic et al., 1987). Treatment of mares with progestins as a prophylaxis for early embryonic loss is therefore common in equine reproduction, however, the effects of this treatment have been questioned (Allen, 2001). The concerns are in agreement with studies which showed that neither mean progesterone concentration was reduced in a group of mares that underwent early pregnancy loss (Papa et al., 1998) nor that early pregnancy loss rate was affected by substitution of different exogenous progesterone doses to ovariectomized embryo recipient mares (Knowles et al., 1993). However, irrespective of species any studies investigating pregnancy or pregnancy loss rates as end points may reveal conflicting results. These are caused by timing of treatment, lack of sufficient animal numbers or the fact that only a proportion of animals with inherently low progesterone concentration may benefit from the respective treatment (Rizos et al., 2012). Nevertheless, when investigating end points that may be directly affected by the treatment, i.e. down-regulation of endometrial progesterone receptors (Willmann et al., 2011b) or early fetal growth rate in aged mares (Willmann et al., 2011a), benefits of oral progestin supplementation during the early post luteal phase could be proven for the horse.

For prevention of early pregnancy loss, stimulation of endogenous progestin secretion has attracted interest in several species. Positive effects of human chorionic gonadotrophin (hCG) treatment during the early luteal phase on corpus luteum function and conceptus development have been suggested in cattle (for review: Lonergan, 2011; De Rensis et al., 2010). Treatment with hCG on day 5 after ovulation increased plasma progesterone concentration via stimulation of luteal tissue in the primary corpus luteum as well as formation of additional corpora lutea in this species (Rizos et al., 2012). As mentioned above, effects of such treatments on pregnancy rates are not consistent (Lonergan, 2011) and their economic benefit is therefore questionable. In the present study, we have investigated the influence of treatment with hCG either for induction of ovulation or during the early luteal phase on plasma progestin concentrations and conceptus growth in early pregnant mares. We hypothesized that hCG stimulates luteal function and conceptus development.

2. Material and methods

2.1. Animals

Warmblood mares (mean weight 542.3 ± 12.6 kg) belonging to the Brandenburg State Stud at Neustadt (Dosse), Germany, were included into the study. The herd consists of a total of 40 active broodmares. For the experiments, mares were classified as foaling (living foal present), maiden (3 year old mares that had not been bred before) or barren (mares that had been bred during the previous breeding season but did not become pregnant or subsequently lost their pregnancies). Until the middle of May, mares were kept inside in groups (8 to 10 animals) and fed oats (3 kg per day) and minerals thrice daily, hay ad libitum. Every day they spent several hours in outdoor paddocks. Beginning in May, the mare groups were kept on pasture all day and in a spacious group stable at night. They were fed oats (2 kg per day) and minerals twice daily and hay ad libitum at night. They had always access to water. Experiments were performed in agreement with German animal welfare legislation and approved by the Brandenburg State Ministry for Rural Development, Environment and Consumer Protection (reference numbers: 23-2347-A-5-1-2010, V3-2347-A-5-1-2013).

2.2. Breeding management

Mares were teased for determination of oestrous behaviour with a stallion three times per week. When the mares showed signs of oestrus or were at the expected time of first postpartum oestrus (approximately 6 days after foaling), their ovaries were examined for the presence of follicles and the uterus for the presence of endometrial oedema by rectal palpation and scanning with a 7.0 MHz linear ultrasound scanner (DP-6600Vet, Mindray, Shenzhen, China). When a moderate or severe uterine oedema and a preovulatory follicle of 35 mm diameter were detected, mares were artificially inseminated with semen from stallions (fresh or cooled-stored) chosen by the stud farm director. If required for management reasons (limited availability of semen on weekends and public holidays), ovulation was induced with human chorionic gonadotrophin (hCG, 1500 IU i.v., Ovogest 300 ad us. vet., MSD Animal Health, Unterschleissheim, Germany) as soon as the preovulatory follicle reached a size of 35 mm in diameter and endometrial oedema was detectable. All insemination doses met the quality criteria defined by World Breeding Federation of Sport Horses (2014). If no ovulation occurred within 48 h after insemination, the mare was inseminated in 48 h-intervals until ovulation was detected (day 0; preovulatory follicle no longer detectable, identification of a corpus haemorrhagicum) with semen of the same stallion. In mares artificially inseminated with frozen semen, transrectal ultrasound of the preovulatory follicle was performed at 8-h-intervals until detection of ovulation and the mare was inseminated as soon as ovulation was detected.

2.3. Experimental design

2.3.1. Experiment 1

In Experiment 1, the influence of induction of ovulation with hCG on progestin secretion, corpus luteum size and conceptus development was investigated. Data from two breeding seasons were included and retrospectively analysed. Management of mares was performed as described above. If considered necessary by the veterinarian responsible for breeding management, ovulation was induced with human chorionic gonadotrophin (hCG, 1500 IU i.v.) as soon as the preovulatory follicle reached a size of 35 mm in

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