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The effects of intrauterine infusion of peanut oil on endometrial health, salivary cortisol and interovulatory period in mares

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ABSTRACT

Intrauterine infusion of peanut oil at Day 10 post-ovulation has been reported to prolong dioestrus in mares. However, the effects of peanut oil treatment on the endometrium and whether the technique is painful have not been assessed. The objectives of this study were, (i) to determine the effect of intra-uterine infusion of peanut oil on endometrial health, (ii) to determine whether use of intrauterine peanut oil is painful and (iii) to confirm that peanut oil causes prolonged dioestrus. Six mares aged 3–12 years old were used in a cross-over design with each mare administered both 1 ml of intrauterine peanut oil and a sham treatment on different oestrous cycles. The effect of intrauterine infusion of 1 ml peanut oil or sham treatment were measured using interovulatory period, uterine fluid accumulation as determined by transrectal ultrasonography, serum progesterone levels, endometrial Kenney biopsy scores and histological features, endometrial eosinophil numbers and salivary cortisol measurements. The individual mare response to intrauterine infusion of peanut oil was variable. Peanut oil infusion did not statistically prolong the luteal phase, nor elevate salivary cortisol levels but did cause superficial erosion of the endometrial surface epithelium in all mares and significantly increased eosinophil numbers in the endometrium ($P = 0.0068$). The Kenney grade for biopsies from 2/6 mares worsened transiently following infusion. In conclusion, intra-uterine peanut oil does not statistically increase the duration of the luteal phase but results in an inflammatory response and increase in endometrial eosinophil numbers suggesting treatment may be associated with a hypersensitivity-type reaction. Those contemplating using peanut oil to suppress oestrus should also be aware of the legislative and regulatory implications.

1. Introduction

Oestrus-related behavioural issues in mares can disrupt athletic performance [1–6]. Altrenogest (Regumate Equine[1]) is probably the drug most commonly used to suppress oestrus in mares. Internationally, its use in mares is not allowed by some governing bodies (e.g. the British Horseracing Authority [7]), but is allowed by others (e.g. the FEI, under certification, [8]; New South Wales Racing [9], and the Hurlingham Polo Association [10]). However, the use of Regumate Equine[1] is not unproblematic since it has the potential to cause positive drug test results for in-contact horses via feed contamination [11], and poses risks to pregnant women, women of childbearing age, and those with certain types of tumour and thrombo-embolic disease. Furthermore, it requires daily administration, which can be burdensome to some commercial operations.

Injectable Altrenogest may provide reliable, short-term suppression of the behavioural signs of oestrus, and avoid some of the problems associated with handling the oral product [6,12]. Such a product (Readyserv[2]) is currently licensed in Australia. The use of medroxyprogesterone acetate (MPA) has been shown to be

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ineffective in suppressing oestrus in mares [3,12,13]. Repeated injections with low dose intravenous [14] or high dose intramuscular [15] oxytocin prolongs dioestrus (thereby suppressing oestrus) in up to 70% of mares. However, protocols require daily injections for 7–29 days [14,15] which is challenging for some owners, with some additionally considering the protocol a welfare concern. Injection of human Chorionic Gonadotrophin during dioestrus also potentially prolongs dioestrus, but has only been assessed in a small number of mares [16]. Gonadotrophin releasing hormone (GnRH) vaccines (reviewed in Ref. [4]) can be effective in suppressing oestrus [5,17]. However, there is individual variation in response to treatment with some (particularly older mares) requiring repeated vaccinations, and other mares entering prolonged (>12 months) suppression of reproductive cyclicity [4,17]. This may be undesirable in a commercial context, particularly if the owner wishes to breed the mare immediately following retirement from competition.

Reports of non-medicinal methods of oestrus suppression include the insertion of a marble into the mare's uterus [18–20], manual disruption of an early embryo (to induce pseudopregnancy) [2]; and, anecdotally, covert ovariectomy. Intrauterine marbles suppress oestrus unreliably [19,20] have been reported to fracture [21], to be associated with colic [22] and can damage the endometrium, impacting upon future fertility. There are also ethical issues associated with failure to declare the insertion of an intrauterine marble, during competition, or at sale. Establishing pregnancies in order to kill the embryos is unlikely to be viewed by the general public as ethically acceptable practice [2]. Ovariectomy not only renders the mare irreversibly infertile, but also surgical risks which may be difficult to justify in an ethical harm:benefit analysis, particularly since ovariectomy does not always abolish oestrus behaviour [23].

In 2011, intrauterine infusion of fractionated coconut oil or peanut oil at Day 10 post-ovulation was reported to cause prolonged dioestrus in mares [24]. Potentially, this method of oestrus suppression has the advantages of not requiring medical treatment at the time of competition; of being non-painful; of not carrying drug-associated risks to in-contact humans or horses, and not causing long-term disruption to the reproductive cycle.

Peanut oil is a more probable candidate for oestrus suppression via prostaglandin synthesis regulation than coconut oil, since peanut oil is comprised of mono- and poly-unsaturated fatty acids (PUFAs) [25], whereas coconut oil is comprised primarily of saturated fatty acids [26]. Notably, the second most abundant fatty acid in peanut oil is omega-6 PUFA, linoleic acid, which has been shown to modulate prostaglandin synthesis and influence the relative production of PGF and PGE in ruminant endometrial cells. If these observations in ruminants are applied to mare endometrial cells, it is possible that exposure of equine endometrial cells to linoleic acid could decrease the synthesis of PGF and subsequently inhibit luteolysis [27]. Anecdotally, peanut oil is being used in clinical practice as a method of oestrus suppression in mares, following the publication of the paper of Wilsher and Allen in 2011 [24]. However, a 2016 paper [28] showed that intrauterine coconut oil causes an inflammatory reaction in the endometrium, which raises the possibility that treatment with intrauterine plant oil can have a detrimental effect on endometrial health and subsequently future fertility. Furthermore, no studies have been reported assessing whether the intrauterine infusion of either coconut or peanut oil is painful for mares. This paper therefore aimed to investigate the clinical suitability of intrauterine administration of peanut oil as a reversible, welfare-friendly and ethical method of oestrus suppression in mares. The objectives of the study were (i) to determine the effect of intrauterine infusion of peanut oil on endometrial health, (ii) to determine whether use of intrauterine peanut oil is painful and (iii) to confirm that peanut oil causes prolonged dioestrus.

2. Materials and methods

2.1. Mares

All animal work was performed in accordance with the Animals (Scientific Procedures) Act 1986 guidelines set by the Home Office and Ethics Committee of the Royal Veterinary College (PPL 70/8577). Six mares were identified as being suitable for inclusion in the study following a clinical reproductive examination, and grading of a screening uterine biopsy sample as Kenney Grade I or IIa. The mares were aged between 3 and 14 years old. Two were Dartmoor ponies (history of donation of multiple embryos); one Standardbred type (no history of foaling); two warmbloods (one maiden, one pluriparous) and one Morgan (who had donated multiple embryos and foaled herself once). The study took place in the physiological breeding seasons across two consecutive years. All mares were kept at grass. Before the start of the experiment, mares were accustomed for 4 days to entering the examination stocks for up to 15 min, to rectal examination, and to having saliva swabs taken (see below), in order to minimise/eliminate the potentially confounding stress which those procedures might cause.

2.2. Study design

All six mares were used according to a cross-over design. For the cortisol and efficacy studies, randomisation of treatment order was included with 3 mares receiving a sham treatment at oestrous one and oil treatment in oestrous two and a further 3 mares receiving oil treatment in oestrous one and sham treatment in oestrous two. For the assessment of endometrial health, all six mares had control biopsies collected at the oestrus prior to both the oestrous periods referenced above. Randomisation for this part of the study was not possible as pre-oil samples were required as controls.

Following initial induction of oestrus by intramuscular injection of 125–250 mcg cloprostenol (Estrumate[3]), each mare had pre-treatment endometrial biopsy samples taken during oestrus (see below for biopsy methods). No further treatments were carried out in the oestrus period in which the pre-treatment endometrial biopsy samples were collected. Having acquired these baseline, pre-treatment endometrial biopsy samples, experiments were undertaken across two subsequent oestrus periods according to the cross over design above.

2.3. Monitoring and manipulation of the reproductive tract

Reproductive status including return to oestrus, ovulation and evaluation of the uterus was monitored by a combination of rectal examination, transrectal ultrasonographic evaluation of the reproductive tract, and biweekly serum progesterone sampling (see below). Biweekly serum progesterone continued throughout the initial post-treatment return to oestrus, subsequent dioestrus, and until subsequent return to oestrus had been demonstrated, up to a maximum of 60 days. Ten days after ovulation, mares received either an intrauterine 'sham' treatment or peanut oil treatment according to the cross over design ($n = 3$ received sham treatment at this first cycle later followed by oil treatment at a subsequent cycle and $n = 3$ received an oil treatment at this first cycle, later to have a sham treatment at a subsequent cycle). The mare was placed in stocks, her rectum manually evacuated, her tail wrapped in a clean rectal glove and bandaged, and her vulva and perineum

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