The effect of hCG, GnRH and $PGF_{2\alpha}$ analogue cloprostenol on the oestrus cycle in jennies

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Abstract

The objectives of this study were twofold. Firstly, the present study was designed to examine susceptibility of the corpus luteum (CL) in early diestrus in jennies; and secondly, to investigate the effect of two commonly used hormonal agents in horses on the induction of ovulation in jennies. The oestrus cycles of eleven jennies were monitored by ultrasound every day. When the dominant follicle reached a diameter of 30 mm, the jennies were treated by intramuscular administration of gonadotropin-releasing hormone agonist lecirelin (GnRH, 50 μg *pro toto*) in the first oestrus cycle, and human chorionic gonadotropin (hCG, 1500 IU *pro toto*) intramuscularly in the second oestrus cycle. Prostaglandin $F_{2\alpha}$ analogue cloprostenolum ($PGF_{2\alpha}$, 0.125 mg *pro toto*) was administered intramuscularly 2 days after the first ovulation and the interovulatory interval was monitored. This study showed that intramuscular administration of 50 μg of GnRH agonist lecirelin resulted in ovulation within 48 h in 73% of treated jennies. Intramuscular administration 1500 IU of hCG was found to be poorly effective to induce ovulation, with 36% of animals ovulating within 48 h. Intramuscular administration of $PGF_{2\alpha}$ analogue cloprostenol 2 days after ovulation was unsuccessful in attempting to shorten the interovulatory interval in donkeys.

Donkey, induction of ovulation, luteolysis

Interest in donkey reproduction has been on the rise recently. Compared to horses, knowledge about management of the oestrus cycle in the donkey is limited. The lack of information about hormonal manipulation of the oestrus cycle in donkeys can seriously reduce the efficiency of assisted reproduction techniques.

The oestrus cycle in jennies lasts 24 days on average (Blanchard et al. 1999; Taberner et al. 2008; Galisteo and Perez-Marin 2010; Quaresma and Payan-Carreira 2015; Díaz-Duran et al. 2017). The length of oestrus is similar to that in mares, while diestrus is longer in donkeys (Vandeplassche et al. 1981; Ginther et al. 1987; Blanchard et al. 1999; Contri et al. 2014). The length of oestrus is in the range of 5.9 ± 2.1 days (Blanchard et al. 1999), 5.64 ± 0.20 days (Taberner et al. 2008) and 6.5 ± 0.6 days (Contri et al. 2014). While the diestrus length ranges from 17.4 ± 2.6 days (Blanchard et al. 1999), 19.83 ± 0.36 (Taberner et al. 2008), and 16.8 ± 0.6 days (Contri et al. 2014). Ovulation usually occurs 1–2 days before the end of oestrus behaviour (Henry et al. 1987, Meira et al. 1995, Blanchard et al. 1999).

The average size of the preovulatory follicle may be breed-dependent. Reported mean diameters of the preovulatory follicles are 44.9 ± 0.5 mm in the Catalonian (Taberner et al. 2008), 43.7 ± 0.13 mm in the Martina Franca donkey breed (Contri et al. 2014), 41.3 ± 1.3 mm in French jennies (Dadarwal et al. 2004) and 36.9 ± 0.7 mm in the "Mexican Burro" donkeys (Díaz-Duran et al. 2017). Follicular dominance is established at a diameter of about 25 mm. Most dominant follicles are about 27 mm in diameter at the onset of oestrus. Follicular growth averages 2.7 mm per day (Dadarwal et al. 2004).

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Synchronization of the jennies' oestrus cycle is possible using exogenous $PGF_{2\alpha}$, primarily as a luteolytic agent to induce oestrus. The earlier oestrus can be induced the better for an effective reproductive management of the jenny. A study of Nigerian jennies established that a double treatment of naturally occurring prostaglandin $PGF_{2\alpha}$ (dinoprost) as tromethamine salt had a better response and therefore was more efficient than a single treatment in oestrus synchronization (Hassan et al. 2017). On the other hand, Carluccio et al. (2008) reported findings which indicate that a single $PGF_{2\alpha}$ R-cloprostenol treatment on day 3 post-ovulation caused the functional regression of corpus luteum (CL) in the jenny, evidenced by both the rapid induction of oestrus and ovulation, and by an abrupt drop in circulating plasma progesterone concentration. They recorded an interovulatory interval of 9.6 days, indicating a marked reduction in the oestrous cycle length. Shortening the oestrus cycle has important implications for reproduction management programs in which insemination needs to be accurately timed (Carluccio et al. 2008). These results are contrary to the notion that the CL is insensitive to prostaglandins in the first 5 days after ovulation. In recent studies in horses, it has been observed that cloprostenol treatment during the post-ovulatory period (0–2 days) induces a decrease in progesterone concentrations over days 5–7 post-treatment, followed by the recovery of progesterone levels and a normal diestrus stage (Troedsson et al. 2001; Brendemuehl 2002; Nie et al. 2003). These findings suggest that cloprostenol could cause luteal ''injury'' or incomplete lysis when administered in early diestrus (Troedsson et al. 2001; Brendemuehl 2002; Nie et al. 2003) and could reflect the still incomplete susceptibility of CL such that it is only capable of a partial response to treatment. There is a need for further investigation focussing on CL responsiveness to $PGF_{2\alpha}$ given to donkeys in early diestrus.

The hormonal agents commonly used for the induction of ovulation in mares are human chorionic gonadotropin (hCG) and gonadotropin-releasing hormone (GnRH). The efficacy of hCG and GnRH for inducing ovulation in jennies has been reported by a few authors (Carluccio et al. 2007; Camillo et al. 2014). Human chorionic gonadotropin is a hormone the biological activity of which is similar to the luteinizing hormone (LH). The effects of hCG in hastening ovulation in mares has been previously studied, and the results revealed that the duration of oestrus and the treatment to ovulation interval was significantly reduced (Barbacini et al. 2000). However, it is well known that the effectiveness of hCG is reduced by successive injections in mares (Duchamp et al. 1987). An alternative method for inducing ovulation in mares is the use of GnRH agonists, such as buserelin, deslorelin, or lecirelin. Carluccio et al (2007) reported that even if hCG administration is the most efficient method for prediction and synchronization of ovulation, current results indicate that, unlike in the mare, even a single administration of lecirelin (GnRH-analogue) can successfully hasten ovulation in jennies where a follicle equal to larger than 30 mm has been detected. The study from Camillo et al. (2014) showed the possibility of inducing ovulation in jennies between 24 and 48 h with a single subcutaneous injection of a very low dose of GnRH agonist buserelin. The effect of a single administration of GnRH analogue lecirelin in the jenny differs from that reported in the mare (Carluccio et al. 2007). In mares, several studies demonstrated the inefficacy of only one or two administrations of GnRH (Duchamp et al. 1987; Camillo et al. 2004).

The aim of this study was to evaluate the response of CL to a single dose of $PGF_{2\alpha}$ analogue cloprostenol administered 2 days after ovulation. The second aim was to evaluate the effect on induction of ovulation by a single intramuscular (i.m.) dose of a GnRH analogue lecirelin in comparison with a single i.m. dose of hCG.

Materials and Methods

This study was carried out from May to August 2018 at the Equine Clinic, University of Veterinary and Pharmaceutical Sciences Brno. The experimental design was approved by the Ministry of Education, Youth and Sports (MSMT-11921 / 2017-5).

Eleven jennies without previous hormonal therapy, 4–15 years old, were successively presented for this trial. All jennies were observed as being clinically healthy, in good body condition, and were kept in individual boxes with open paddocks allowing freedom of movement throughout the whole trial. Feed with hay and water was provided *ad libitum*. Daily trans-rectal ultrasonographic observations were done for the detection of follicular dynamics. Detection of oestrus in jennies was done by teasing with a donkey stallion (mouth clapping, urination, vulvar activity). In the first oestrus cycle, once there was evidence of external symptoms of oestrus and of follicular diameter 30 ± 1 mm, all jennies were administered the GnRH agonist lecirelin i.m. (50 µg *pro toto*, Supergestran, Fatro, Bologna, Italy). Ovulation was monitored daily by ultrasonographic observation. The second day after ovulation $PGF_{2\alpha}$ cloprostenolum i.m. (0.125 mg *pro toto*, Oestrophan, Bioveta, Ivanovice n. Hané, Czech Republic) was administered and the jennies continued to be monitored daily. In the second oestrus cycle, once there was evidence of external symptoms of oestrus and of a follicular diameter of 30 ± 1 mm, all jennies were administered hCG i.m. (1500 IU *pro toto,* Pregnyl, NV Organon, Oss, The Netherlands) and were also monitored daily until ovulation.

Results

The results are presented in Table 1. A positive response (ovulation within 48 h) in jennies injected with GnRH agonist was detected in 8 out of 11 jennies (73%). A positive response in jennies injected with hCG was detected in 3 out of 11 jennies (27%). A positive response in jennies injected with $PGF_{2\alpha}$ (shortening of diestrus to less than 16 days) was detected in 4 out of 11 jennies (36%).

Table 1. Results from daily examinations in jennies.

Jenny	FD GnRH (mm)	OV GnRH (until hours)	FD OV 1 (mm)	IOV (days)	FD $PGF_{2\alpha}$ (mm)	FD hCG (mm)	OV hCG (until hours)	FD OV 2 (mm)
1	32 × 34	96	35 × 38	8	< 20	33 × 33	anovulatory follicle	-
2	33 × 37	72	30 × 40	20	< 20	33 × 38	48	32 × 39
3	33 × 33	48	32 × 35	5	< 20	32 × 32	120	39 × 42
4	30 × 33	120	31 × 35	18	< 20	30 × 33	anovulatory follicle	-
5	30 × 35	48	31 × 38	21	< 20	30 × 35	72	40 × 44
6	30 × 31	48	33 × 35	26	< 20	30 × 31	96	34 × 34
7	31 × 32	24	32 × 35	22	< 20	30 × 30	48	33 × 36
8	32 × 34	48	34 × 36	19	< 20	32 × 34	72	31 × 36
9	33 × 35	24	33 × 35	16	< 20	30 × 32	48	30 × 36
10	31 × 32	48	34 × 37	8	< 20	31 × 33	120	35 × 36
11	32 × 34	48	30 × 38	10	< 20	31 × 34	96	33 × 33

FD GnRH – follicular diameter on time of administration GnRH; OV GnRH – ovulation after GnRH administration; FD OV 1 – follicular diameter before ovulation; IOV – interval between ovulation and administration of hCG; FD $PGF_{2\alpha}$ – follicular diameter on time of administration $PGF_{2\alpha}$; FD hCG – follicular diameter on time of administration hCG; OV hCG – ovulation after hCG application; FD OV 2 – follicular diameter before ovulation

Discussion

In mares there is a long-standing belief that an early developing CL (< 5 days after ovulation) is not susceptible to $PGF_{2\alpha}$ (Lofstedt 1988). However, Bergfelt et al. (2006) discovered that a single dose of native prostaglandin $PGF_{2\alpha}$ treatment on the 3rd day after ovulation resulted in structural and functional regression of the CL and hastened the interval to the next ovulation, despite post-treatment resurgences in progesterone in mares. The CL appears to be mature on the 3rd day post ovulation also in jennies. Injection of $PGF_{2\alpha}$ R-cloprostenol at this time induces regression of the CL and a return to oestrus (Carluccio

et al. 2008). There is a need for examination of the luteolytic effect of $PGF_{2\alpha}$ as earliest after ovulation as possible.

In this study, 0.125 mg of cloprostenolum was administered i.m. 2 days after ovulation to investigate the susceptibility of CL in early diestrus. Shortening the interovulatory interval of the jenny may be useful in reproductive management, because diestrus in donkeys is longer than in horses. An earlier onset of oestrus was observed in 4 jennies from 11 (36%). It seems that the partial failure to hasten oestrus and ovulation with a $PGF_{2\alpha}$ treatment 2 days after ovulation was due to a partial or incomplete luteolysis and resurgence of progesterone. Nie et al. (2003) reported that repeated administration of cloprostenol over 24 h in the early post-ovulatory period in mares may more effectively impair the luteal function than a single dose. In a recent study of Nigerian jennies, it was also established that the double treatment of naturally occurring prostaglandin $PGF_{2\alpha}$ (dinoprost) had a better response in oestrus synchronization (Hassan et al. 2017), but they did not mention when it was administered relative to the time of ovulation. It was suggested that a double treatment should be administered as early as 48 h after ovulation.

Induction of ovulation is another essential step in assisted reproduction. Accurate prediction of ovulation is invaluable for artificial insemination in donkeys as in horses. In a previous study, Carluccio et al. (2007) reported that, unlike in mares, even a single administration of lecirelin (GnRH-analogue) can successfully hasten ovulation in jennies. In their study a dose of 100 µg of lecirelin was administered intravenously (i.v.). We found it much easier to administer i.m. instead of i.v. due to the donkey's behavioural issues. Jennies with follicles equal to or larger than 30 mm were treated with either lecirelin or hCG i.m. In this study, 50 µg of lecirelin *pro toto* were administered i.m. and positive reactions (ovulation within 48 h) were detected in 73% of the monitored jennies. These findings suggest that a lower single dose and an easier form of application of the GnRH analogue lecirelin can also successfully hasten ovulation in jennies.

Another result from the present study showed the ineffectiveness of hCG administered i.m. (1500 IU *pro toto*). In a recent study, Carluccio et al. (2007) treated jennies with hCG with a single i.v. injection of 2500 IU hCG in two subgroups according to the follicular diameter (subgroup 30–35mm and subgroup 36–40 mm). Ovulation occurred in the subgroups within 48 h in 92% and 100%, respectively. This suggests that the follicular diameter at the time of administration being smaller than 35 mm was probably not the cause of failure. In order to shorten the interval to ovulation, we administered hCG i.m. at a follicular diameter > 30 mm and expected ovulation within 48 h. Carluccio et al. (2007) suggested that the smaller follicles require a longer interval to ovulation compared to larger follicles, at the time of administration of hCG. Precise timing of ovulation induced by hormonal agents in jennies needs further investigation. Different methods of application, chosen due to behavioural issues, could be the reason. Different pharmacokinetics in donkeys have been described (Matthews et al. 2001; Matthews et al. 2002). In comparison with a dose of 2500 IU hCG i.v. when almost 100% ovulation was achieved (Carluccio et al. 2007), the dose of 1500 IU administered i.m. was essentially ineffective and will need to be further optimized. There are several reports that showed reduced efficacy of hCG after repeated use in the same season (Wilson et al. 1990; McCue et al. 2004). The use of GnRH agonists as an alternative method for inducing ovulation in jennies was considered.

In conclusion, this study demonstrated that a single dose 50 µg of GnRH analogues lecirelin administered i.m. was able to induce ovulation in jennies within 48 h. The dose of 1500 IU of hCG administered i.m. proved to be poorly effective, with only 27% of animals ovulating within 48 h. A single administration of the synthetic $PGF_{2\alpha}$ analogue cloprostenol 2 days after ovulation is not successful for shortening the interval to the next preovulatory follicle in jennies.

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