Comparative efficacy of BioRelease Deslorelin® injection for induction of ovulation in oestrus mares: a field study

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Objective To investigate the comparative efficacy of BioRelease Deslorelin® (BRD) and Ovuplant® for induction of ovulation in cyclic mares in Australia.

Methods Ovarian follicular activity of 60 mares for a total of 95 cycles was monitored by ultrasonography until they developed a follicle ≥30 mm and a uterine oedema pattern of 3. Mares were then randomly allocated to one of three treatment groups: (1) treatment with 1.25 mg BRD, (2) a single Ovuplant pellet or (3) 1 mL compound sodium lactate control. Follicular activity was monitored with ultrasonography every 12 h until ovulation was detected or for at least 5 days post treatment. The injection site on each mare was monitored for reaction for a minimum of 5 days post treatment.

Results There was no difference in the percentage of mares ovulating within 48 h when treated with BRD (93.75%) compared with Ovuplant (87.09%). Treatment with both ovulating agents significantly decreased the time to ovulation compared with control mares ($P < 0.00005$). More mares had injection site reactions with Ovuplant (64.5%) treatment compared with BRD (15.6%) or control mares (0%) ($P < 0.00005$).

Conclusions Treatment of mares with 1.25 mg BRD when there is a follicle ≥30 mm and uterine oedema pattern of 3 is as effective as treatment with Ovuplant.

Keywords breeding programs; horses; oestrus; ovulation agents

Abbreviations BRD, BioRelease Deslorelin®; GnRH, gonadotrophin-releasing hormone; hCG, human chorionic gonadotropin

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The use of ovulating agents has become an integral part of modern equine reproductive practice. The ability to induce ovulation at a predictable time has increased the efficiency of equine breeding programs. Benefits include a decreased number of services per cycle for popular stallions, allowing larger books of mares annually; scheduled breeding for mares transported to stallions; fewer breedings per cycle, resulting in less uterine contamination (particularly for mares with delayed uterine clearance or increased susceptibility to uterine infections); insemination close to time of ovulation when using frozen-thawed semen; ensuring adequate intervals between natural services for specific stallions; and allowing synchronisation of donor and recipient mares in embryo transfer programs. Many of these result in reduced labour and veterinary costs while improving efficiency.

Currently, there are three licensed products available in Australia for induction of ovulation: hCG (Chorulon, Intervet Australia, VIC, Aust); deslorelin acetate, available as a controlled- release subcutaneous implant (Ovuplant®, Virbac Australia) and deslorelin acetate in a short-term release biocompatible liquid (BioRelease Deslorelin, Caledonian Holdings Pty [BRD]).

Human chorionic gonadotropin (hCG) is a large glycoprotein that is purified from the urine of pregnant women and acts like luteinising hormone in the mare. It has been shown to predictably induce ovulation when 1500–5000 IU is administered to mares that are in behavioural oestrus with a follicle size of at least 35 mm.[1]

Although the use of hCG in horses has been reported from as early as the 1930s,[2] there are some associated drawbacks, such as its human origin and consequent variations in purity and supply.

Sullivan et al.[3]reported refractoriness after multiple doses of the drug in the same season.[3–5] Further work[6] demonstrated the formation of antibodies to hCG and this was hypothesised to be responsible for the previously reported reduction in efficacy, although there is some debate in the literature around the significance of these antibodies.[4]

Work in Australia in the early 1990s showed that an implant containing the gonadotrophin-releasing hormone (GnRH) analogue, deslorelin, would induce ovulation at a predictable time in mares.[7] Available as an implant, Ovuplant has been shown to reliably induce ovulation in oestrus mares with a follicle >30 mm.[7] Benefits include its small molecular weight, making it less antigenic, its use in alternate cycles to hCG and, because it is a synthetic product, it is not subject to potential viral contamination or disruption in supply.[7]

An increased interovulatory period has been identified as a side effect of the use of Ovuplant.[8–10] It has been suggested that prolonged secretion of deslorelin from the implant results in downregulation of pituitary gonadotropin secretion and subsequent suppression of follicular activity.[11,12] Further work demonstrated that this pituitary downregulatory effect and resultant extended interovulatory period could be avoided by implant removal once the mare has ovulated.[13–15] To facilitate removal, some practitioners favour implantation in the vulva instead of the neck, which is the manufacturer's recommended site.[5]

Controlled-release formulations of deslorelin acetate have been available as compounded products from various sources in Australia and the USA for many years.[16] However, BRD has recently been registered and brought to market in Australia. This preparation is in a liquid form and allows 1.25 mg of deslorelin acetate to be administered by intramuscular injection. The extended interovulatory

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intervals recognised with the use of Ovuplant have not been seen with the use of other biorelease formulations.[16,17]

In this study we compared the efficacy of the recently approved product BRD for induction of ovulation in oestrus mares to that of Ovuplant and control untreated mares. As this study formed part of the application for registration of BRD, we also monitored and compared injection sites to fulfill the requirements for drug registration approval.

Materials and methods

The experimental animals were part of a herd of Thoroughbred and Standardbred mares maintained as recipient mares for an embryo transfer program. The mares ranged in age between 3 and 15 years (mean age, 7.6), were reproductively sound and in good body condition. They were maintained on pasture and fed lucerne hay and grain supplement as necessary. The study was conducted during January and February 2013 at Goulburn Valley Equine Hospital, in north-eastern Victoria, Australia.

Each mare's reproductive tract was evaluated by rectal palpation and ultrasonography (Mindray 6600Vet, with 5-Mhz probe). The mares were examined two or three times weekly until they had evidence of a corpus luteum with a maximum follicle size <25 mm; oestrus was then induced by luteolysis using an intramuscular injection injection of the prostoglandin F2α analogue, closprostenol (250 µg, Ovuprost, Bomac Animal Health Australia, NSW, Aust). Mares were subsequently examined at intervals of 1–3 days as dictated by an experienced operator until they displayed ultrasonographic evidence of oestrus, with a dominant follicle ≥30 mm diameter, a uterine oedema pattern of 3[18] and softening of the cervix. Candidates (n = 60) were then randomly assigned to one of three groups using an online random number generator: BRD group (n = 32): 1.25 mg BRD as an IM injection; Ovuplant group: (n = 31): SC implant containing 2.1 mg deslorelin; and Control group: (n = 32): IM injection of 1 mL compound sodium lactate solution (Baxter Healthcare, NSW, Aust).

Both BRD and compound sodium lactate were injected using a sterile disposable 3-mL syringe and a sterile disposable 20G needle. Ovuplant implants were administered using the sterile single-use applicator supplied by the manufacturer. All treatments were administered in the neck.

Once treated, mares were examined by rectal palpation and transrectal ultrasonography every 12 h until ovulation was confirmed or until 5 days post treatment. Ovulation was diagnosed by the absence of the previously identified pre-ovulatory follicle and ultrasonographic visualisation of the characteristic echogenic structure formed by the collapse of the follicular wall.[18] Ovuplant implants were not removed, because that is not indicated by the manufacturer's directions for use that accompany the product. To fulfill drug registration approval requirements, all injection sites were examined for any evidence of reaction at each reproductive examination and once the mare had ovulated the injection site was examined daily for a total of 5 days.

Table 1. Time of ovulation for mares treated with Ovuplant® or BRD

	Ovulated within 48 h (%)	Before 24 h (%)	24–48 h	> 48 h
Control (n = 32)	7 (21.9)			
Ovuplant® (n = 31)	27 (87.1)	2 (6.5)	25 (80.6)	4 (12.9)
BRD (n = 32)	30 (93.8)	0	30 (93.8)	2 (6)

BRD, BioRelease Deslorelin®

Statistical analysis

Generalised linear regression analysis was used to test for significance between treatments for mares ovulating within 48 h of treatment, injection site reactions and duration of injection site reactions. The percentage of mares ovulating in the same 24-h period was analysed using Fisher's exact test. Comparison of follicular size at treatment was done using Student's t test. Contingency table analysis was also used to confirm the significance of differences in lesion scores between the control and treatment groups.

Results

There was a significant difference ($P < 0.001$) between the Control group and the BRD and Ovuplant groups in the number of mares that ovulated within 48 h of treatment (Table 1). There was no significant difference between the BRD and Ovuplant groups for the number of mares ovulating 24–48 h post treatment ($P > 0.05$).

The mean follicular diameter at treatment was 33.91 mm (SD ± 2.44) for the Control, 33.47 mm (SD ± 1.93) for the BRD and 33.35 mm (SD ± 2.20) for the Ovuplant groups. There was no significant difference ($P > 0.05$) between the groups when follicular size at treatment was compared using Student's t test.

A significant difference ($P < 0.00005$) was found in the number of injection site reactions between treatment groups, with the Ovuplant group (20/31, 64.52%) having a higher incidence than the BRD group (5/32, 15.6%). The time taken for injection site reactions to regress was greater in mares treated with Ovuplant, with a median duration of 72 h compared with 36 h for BRD-treated mares ($P < 0.001$). The reactions seen were typically mild to moderate swelling of the injection site and sometimes these swellings appeared to be painful on palpation.

Discussion

This study demonstrated an equivalence in efficacy between BRD and Ovuplant in inducing ovulation in oestrus mares with a dominant follicle ≥30 mm in diameter. The treatments were administered when the follicle diameter was ≥30 mm in oestrous mares, as this is the labelled instruction on the BRD and Ovuplant products. In our study, 87.1% of mares treated with Ovuplant ovulated within 48 h, which is in line with other reports.[7,19] Others have independently reported 100% ovulation rates with Ovuplant;[20,21] however, differences in recruitment criteria resulted in mares being treated with larger follicles (>40 mm) and variations in breed may account for

difference in the results. We had 93.8% of mares treated with BRD ovulate within 48 h of treatment, which is in agreement with another study using a controlled-release product that reported an ovulation rate of 89.9% at 48 h.[22] Others reported an ovulation rate of 68.8% 48 h after treatment with deslorelin, although it is not clear from that report whether the authors used a similar controlled-release product.[23] We observed a peak in ovulation 24–48 h after treatment for both deslorelin products, similar to previous studies using Ovuplant[20,21] or other injectable biorelease formulations of deslorelin.[22]

Reports in the late 1990s identified a side effect of an increased interovulatory interval in some mares treated with implant formulations.[8,9] It was later suggested that the increase in interovulatory period was the result of pituitary downregulation.[11,12] Advances in biocompatible short-term release technology saw injectable forms of deslorelin become available via compounding in the USA and Australia, subsequently leading to the registration in the USA of a controlled-release deslorelin product by the Food & Drug Administration in 2010[24] and of BRD by Australian authorities in 2013. To date there are no reports of increased interovulatory interval when using the BRD formulation. Unfortunately, in our study, we were unable to observe the next oestrous cycle to identify any differences in interovulatory intervals associated with the products used in the study group of mares.

There were no serious adverse reactions to any of the treatments administered during the trial, although more mares treated with Ovuplant had injection site reactions compared with the BRD-treated mares and control mares. The Ovuplant implant itself may induce a local inflammatory response or reactions may be a result of the large needle used to insert the implant, or a combination. During this study both BRD and Ovuplant treatments were injected without undue resentment from most of the mares.

In summary, intramuscular administration of BRD at the labelled dose is as effective as an Ovuplant implant for the induction of ovulation in oestrous mares with a dominant follicle diameter of ≥30 mm.

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