Effect of fluorogestone acetate (FGA) sponge with oestradiol benzoate (OB) on oestrus response and behaviour in Dorper cross ewes

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Abstract

The purpose of this experiment was to identify the most suitable oestrus synchronisation protocol using 30 mg fluorogestone acetate (FGA) sponges with oestradiol benzoate (OB) for sheep. Thirty-four Dorper crosses ewes, randomly allocated into three groups. Ewes in each group are either received FGA with i) corn oil (Control; n=11); ii) 0.05 mg OB (OB-L; n=11) or iii) 0.25 mg OB (OB-H; n=12). A day before FGA removal, one ml of prostaglandin (PG) was injected into each ewe regardless of their treatment groups. Oestrus behaviours observation was conducted with the involvement of four alternate fertile rams after 24 hours post-FGA removal and OB injection. Data of oestrus response and behaviour was analysed using one-way ANOVA. Results showed that all ewes in OB-L and OB-H groups had oestrus within 60 hours of observation, while only 36.4% in the Control group. Meanwhile, ewes with standing oestrus in OB-L and OB-H groups had a shorter interval compare to Control group. Moreover, ewes in OB-L and OB-H groups showed a significantly higher ($p<0.05$) percentage of ewes express their oestrus behaviour compared to ewes in Control group. The results suggested that synchronisation using intravaginal FGA with OB either low or high dose improve the oestrus response of Dorper crosses ewes compared to synchronisation with non-administration of OB.

Keywords: oestrus synchronisation, oestradiol benzoate, oestrus response, fluorogestone acetate, Dorper cross ewe

Introduction

Oestrus synchronisation (OS) in sheep has been implementing worldwide. It has been introduced in sheep breeding programmes by manipulating the ewe's oestrous cycle thus enabling a large number of ewes to be mated with the ram (Martemucci & D'Alessandro, 2011; Silva *et al.*, 2020). This approach is widely implemented in farms due to its well-known positive result and increases the lambing rate and subsequently improve farm management by allowing lambing activities to be carried out almost at the same period and produce better results regarding oestrus and pregnancy rate (Silva *et al.*, 2020; Wei *et al.*, 2016). The researchers have optimized different OS protocols that suit the best for each sheep breed, however further information is needed for those breeds that are not well established. Information regarding the application of FGA in sheep especially for the Dorper breed reared in Malaysia is lacking. There are different protocols of OS that have been introduced such as administration of prostaglandins, melatonin, progesterone and its analogues (Fierro *et al.*, 2013; Santos - Jimenez *et al.*, 2020; Uslu *et al.*, 2012).

However, different OS protocols in sheep may illustrate different results not just due to differences in breed, size or age of animals. The factors such as management, nutritional and body condition of animals, as well as the route and the interval of hormone administration may affect the OS which subsequently would influence the fertility of female animals (Santolaria *et al.*, 2011). Apart from that, the application of FGA pessary together with frequent use of equine Chorionic Gonadotropin (eCG) may cause the development of anti-eCG antibodies (Hervé *et al.*, 2004). These occurrences have been seen in cattle (Rizzo *et al.*, 2009) after continuous administration of eCG during the oestrus synchronisation regime. In contemplation of eCG, the OB hormone is introduced as a substitute for eCG usage in OS. In addition, it has been reported that OB can induce luteinizing hormone (LH) and ovulation subsequently increase fertility in female animals (Karimi *et al.*, 2007). Thus, this study was conducted to evaluate the oestrus response and behaviour of Dorper cross ewes synchronised with 30 mg FGA sponge, PG and with different doses of OB.

Materials and Methods

Experimental area and Animals

The study was conducted at Sumobek Farm, Kuala Pilah, Negeri Sembilan, Malaysia. The farm geographical area is a hilly site and the feeding system implemented for the experimental animal is a cut and carry system. Thirty-four non-pregnant Dorper cross ewes age between 2 to 4 years, body condition score within 2.5 to 4 (scale 1-5) with bodyweight range from 20 to 40 kg were used. All ewes were fed with chopped corn stover, soybean curd waste and commercial concentrate based on the normal practice of the farm management.

Experimental design

Ewes were randomly assigned to three synchronisation groups that received intravaginal 30 mg FGA sponge for 7 days either with: i) 1 ml corn oil (Control; n=11); ii) low dose, 1 ml of 0.05 mg/ml OB (OB-L; n=11) or iii) high dose, 1 ml of 0.25 mg/ml OB (OB-H; n=12). Later, all ewes were administered with prostaglandin (PG: 1 ml of 250 µg/ml cloprostenol) on day 6 and different concentrations of OB on day 7 (day FGA removal). Approximately 24 hours after FGA removal, four selected fertile rams were alternately released into each group for oestrus detection and subsequently mating. Observation on oestrus behaviour or sign were conducted at every 4 hours for 30 minutes until 72 hours after OB administration. The number and time of ewes were observed with different behaviour or sign were recorded. The details of the oestrus behaviour and signs were referred to Katz and McDonald (1992).

Statistical Analysis

Data collected were analysed using the Analysis of variance (ANOVA) and Chi-square test of SAS 9.4 software. Analysis tests with a confidence interval of 95% and $p<0.05$ were considered significantly different.

Results and Discussion

All (100%) Dorper cross ewes in both OB groups were showed oestrus behaviour and only 36.4% from the Control group. There was a significant difference ($p<0.05$) in the percentage of ewes expressed oestrus behaviours or sign and time interval from OB injection to standing oestrus (Table 1).

The percentage of ewes expressed oestrus sign parameters indicated that ewes synchronised with OB, regardless of OB-L or OB-H, it shows 63% higher than in the Control group. However, no difference in the time interval from OB injection to the onset of oestrus between different OB synchronised groups. Even though there was no significant difference ($p>0.05$), ewes synchronised with OB-L had the shortest interval approximately 3 hours and 8 hours earlier than ewes synchronised with OB-H and Control groups, respectively. Meanwhile, ewes synchronised with OB-L and OB-H groups have shorter time intervals from OB injection to standing oestrus compared to the Control group with approximately 20 hours and 16 hours, respectively.

The result from this experiment showed a similar finding as reported by Lammoglia *et al.* (1998), the cows that received OB during oestrus synchronisation increased the number of animals that exhibited oestrus and also shorten the time interval from pessary removal to the onset of oestrus. During the oestrus period, there will be high oestrogen levels in the blood circulation and it was secreted by preovulatory Graafian follicle (Hafez & Hafez, 2013).

Table 1. The oestrus response and behaviour observed in Dorper cross ewes synchronised with FGA with corn oil (Control), low dose (OB-L) and high dose oestradiol benzoate (OB-H)

Parameters	Control	OB-L	OB-H	P-Value
No. of ewes	11	11	12	
No. of ewes with oestrus	4	11	12	
Oestrus response				
Oestrus incidence (%)	36.4^{b}	100^{a}	100^{a}	<0.0001
Onset of oestrus (h±SE)	32 ± 4.62^{a}	24 ± 0.0^{a}	27 ± 1.98^{a}	ns
Standing of oestrus (h±SE)	48 ± 8.00^{a}	28 ± 1.62^{b}	31.7 ± 2.97^{b}	<0.05
% Oestrus behaviour observed (n)				
Seeking for ram	27.3^{b} (3)	45.5^{b} (5)	100^{a} (12)	<0.001
Tail wagging	27.3^{b} (3)	90.9^{a} (10)	83.3^{a} (10)	<0.001
Nudging	18.2^{b} (2)	54.6^{ab} (6)	72.7^{a} (8)	<0.05
Sniffing	18.2^{b} (2)	81.8^{a} (9)	66.7^{a} (8)	<0.001
Homosexual mounting	0.0 (0)	27.3 (3)	33.3 (4)	ns
Stand to be mounted	18.2^{b} (2)	100^{a} (11)	100^{a} (12)	<0.001
Mating	18.2^{b} (2)	100^{a} (11)	100^{a} (12)	<0.001

%: percentage; n: number of animal; FGA: 30 mg fluorogestone acetate; Control: 1 ml corn oil as a carrier solution; OB-L = 1ml of 0.05 mg/ml oestradiol benzoate diluted in corn oil; OB-H = 1 ml of 0.25 mg/ml oestradiol benzoate diluted in corn oil; h: hour; SE: standard error; ns: Not significant
Superscript ^{ab} showing significant different between column

However, synchronised ewes may also exhibit oestrus behaviour or signs even without the formation of the large follicle. Administration of exogenous oestrogen from OB injection to ewes as part of oestrus synchronisation protocol may suppress the dominant follicle and promote a new follicular wave Alnimer (2005) and Burke *et al.* (2001). Administration of OB was believed to cause luteolytic and autotrophic actions, which subsequently may depress the luteal progesterone production and hastened the interval to the onset of oestrus (Takada *et al.*, 2012). Other than that, the time interval between OB injections to the standing of oestrus among synchronised groups was shorter regardless of OB-L or OB-H groups compared to Control group. The ewes in both OB-L and OB-H groups, may have a sufficient level of oestrogen received from the administration of OB. Subsequently be able to express earlier standing oestrus behaviour compared to ewes in Control group and become receptive to male animals and ready to be mated (Gordon, 2017).

The OB-H group had 54.5% and 72.7% higher of ewes seeking for the ram; 18.1% and 54.5% higher of ewes nudging shows behaviours compared with OB-L and Control groups, respectively. Meanwhile, the OB-L group had 7.6% and 63.6% higher of ewes with tail wagging; 15.1% and 63.6% higher of ewes shows sniffing behaviours compared with OB-H and Control groups, respectively. In addition, ewes that are stand to be mounted and mating behaviour showed by both ewes groups regardless synchronised with OB-L or OB-H have 81.8% higher as compared with Control group. However, there is no significant difference ($p>0.05$) on homosexual mounting between ewes in groups synchronised with different doses of OB.

During the courting period, sexual behaviour expressed by ewes was classified as attractivity, proceptivity and receptivity (Fabre-Nys & Gelez, 2007). Attractivity and proceptivity behaviour was an initiation of mating before copulation, whereas proceptivity and receptivity behaviour was usually expressed by ewes in a short period (Ekiz & Ozcan, 2006). Oestrus behaviour (attractivity and proceptivity) observed frequently expressed by the synchronised ewes was seeking for ram and tail wagging. According to Gordon (2017) and Hafez and Hafez (2013), ewes on heat will seek out and remain near to rams and this has been a strong factor influencing their oestrus behaviour. Moreover, tail wagging expressed by the ewes during the oestrus period is known to attract and spread pheromone to rams. Billings and Katz (1999) reported that administration of oestradiol are able to enhance oestrus in female animals and this had an agreement with this experiment; where ewes administrated with OB regardless of OB-L or OB-H groups had a higher frequency of ewes that exhibit oestrus compared to ewes in group administrated with Control. In contrast, proceptivity and receptivity behaviour usually expressed together by ewes; were stand to be mounted and mating, respectively. Both activities were observed during the observation period in this study. When ewes are ready for mating, stands to be mounted followed physical sign commonly exhibited, which were subsequent mounting and mating by rams (Katz & McDonald, 1992). Only 36.36% of ewes synchronised with Control expressed oestrus signs and this may be due to the absence of OB. The ewes synchronised with Control that do not express any oestrus signs were probably due to no formation of new follicles cause less production of oestrogen in the blood circulation, therefore the ewes will not express oestrus signs (Khanum *et al.*, 2008).

All Dorper ewes in the OB-L group have shown oestrus behaviour at 24 to 36 hours after OB administration ($p<0.05$) compared with Control, but no difference ($p>0.05$) with OB-H (Figure 1)

The length of oestrus or heat in ewes are ranging between 24 to 36 hours (Quirke *et al.*, 1981). Therefore, the oestrus behaviour or signs could still be observed up until 60 hours after the sponge removal. The ewes synchronised with OB-H had shown an increasing number of animals expressed signs of oestrus and reached up to approximately 91.7% from 24 hours to 36 hours of observation. Other than that, ewes in the group synchronised with Control had shown less number of ewes expressed oestrus sign throughout of 60 hours of oestrus observation with a maximum of 18.2% (our out of eleven ewes expressed oestrus signs).

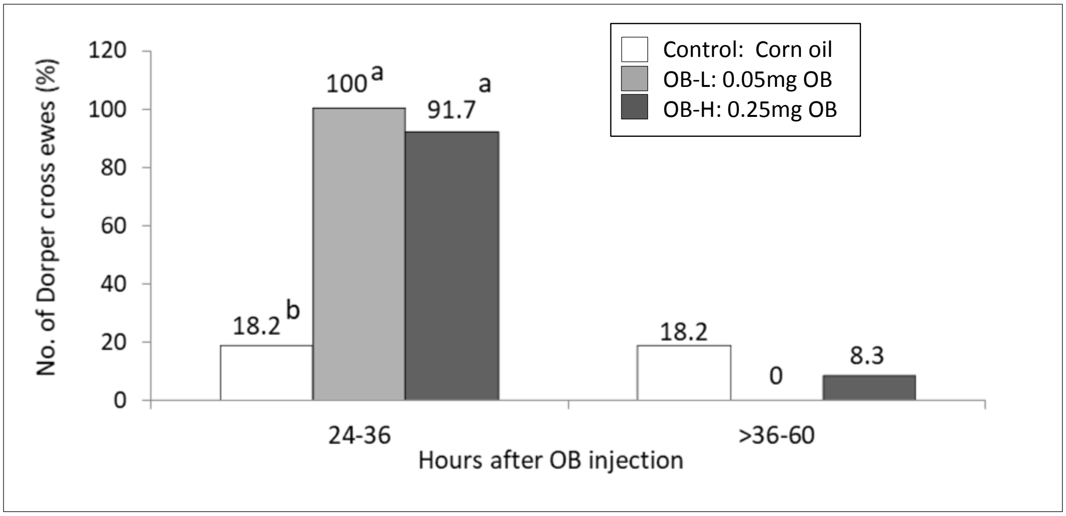

Figure 1. Number of ewes (%) expressing oestrus behaviour or sign at 24-36 and >36-60 hours after OB injection

The synchronisation protocols were considered as compact oestrus (shorter interval) when most of the Dorper cross ewes showed oestrus within the first 36 hours after FGA removal or post-OB administration. There was a significant difference ($p<0.05$) of ewes showing oestrus at 24 hours to 36 hours between the group of ewes synchronised with different OB doses. The Dorper cross ewes in the group synchronised with OB-L (81.8%) and OB-H (73.5%) had more compact of oestrus than ewes in the group synchronised with Control. Meanwhile, there was no significant difference ($p>0.05$) in the occurrence of oestrus behaviour and signs at 36 hours to 60 hours, were observed among the group of ewes synchronised with different OB doses. In this experiment, all synchronised ewes with OB-L showed oestrus signs within the first 36 hours post-OB administration followed by ewes synchronised with OB-H. However, the number and percentage of ewes synchronised with Control with the onset of oestrus within stipulated time were less. Thus, ewes synchronised with OB-L and OB-H was considered to have more compact oestrus (shorter interval) compared to ewes synchronised with Control.

Administration of OB in oestrus synchronisation is known to shorten the interval and variation of onset of oestrus and finally contribute to more compact of synchronisation protocols (Day *et al.*, 2000). Other than OB administration, the presence of rams was believed to affect advancing the timing of preovulatory surge of LH, thus shorten the interval from FGA removal to the first sign of oestrus (Gordon, 2017). This is also had an agreement with this study. The administration of OB resulting in more compact interval between sponge removal and oestrus response compared to the group with non-administrative with OB. Moreover, variation of oestrus response between studies may increase due to several other factors such as breed, age, body condition, management system, nutritional status, exogenous hormone, synchronisation protocols and climate have been reported (Zeleke *et al.*, 2005).

Conclusion

In this experiment, we implement OS with the combination of progestagen and prostaglandin. With the involvement of intravaginal pessary FGA (30mg), PG and OB (OB-L and OB-H), we observed an increase in the orientation of oestrus. A positive outcome was achieved due to the involvement of OB which enhances oestrus orientation in Dorper crosses ewes. This is in agreement with other researchers (Lammoglia *et al.* (1998) and Evans *et al.* (2003)) who showed that OB was able to increase oestrus compared to a normal approach. Furthermore, administration of OB hastens the interval of oestrus thus leading to compact synchronisation and being able to mate the Dorper crosses ewe.

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