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https://dx.doi.org/10.4314/nvj.v43i1.2 **ORIGINAL ARTICLE**

EFFECTS OF REPEATED INJECTIONS OF CLOPROSTENOL SODIUM DURING THE EARLY LUTEAL PHASE ON LUTEOLYSIS AND HORMONAL CHANGES IN LARGE WHITE X PIETRAIN SOWS IN OTUKPO, NIGERIA

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ABSTRACT

This study was carried out to determine the effect of repeated injections of cloprostenol sodium during the early luteal phase on luteolytic capacity and hormonal changes of Large white x Pietrain in Otukpo. A total of ten (n = 10) oestrus Large white x Pietrain sows were randomly divided into two groups. experiment and a control. Group I (n = 5) no synchronizing agent was administered Group II (n = 5) received 6 doses of 500 µg of cloprostenol sodium at days 5, 6, 7, 8, 9 and 10 of the oestrous cycle and was monitored for natural oestrus twice daily from 0700 – 1000 and 1500 – 1800h. The fertility parameters determined in this study were: Time to onset of oestrus (TOO), duration of oestrus (DOO), total litter size (TLS), total born alive (TBA) average body weight (Av. BW). Five (5) milliliters of blood was collected via the posterior vena cava before $PGF_{2\alpha}$ injections on at day 1 and 5, 6, 7, 8, 9, 10 and every other day afterwards till next observed oestrus. The extracted serum from the collected blood was assayed using competitive ELISA for Oestradiol (E_2) progesterone (P_4) concentrations. Variables such as TOO, DOO, TLS, TBA, Av. BW and data on E_2 and P_4 profile were expressed as mean ± SEM. Student t-test and Tukey's post-hoc test were used to compare the percentages and mean values between the groups. A value of $P \leq 0.05$ was considered significant. Results TOO was 484.80 ± 13.99 h, and 484.80 ± 15.92 h; DOO was 86.40 ± 5.88 h and 76.80 ± 4.80 h; TLS was 10.00 ± 0.84 and 10.20 ± 0.66; TBA was 10.00 ± 0.84 and 10.00 ± 0.55 and Av. BW was 1.26 ± 0.09 kg and 1.06 ± 0.05 kg in groups I and II respectively. However, the fertility parameters showed no statistically ($P \leq 0.05$) significant differences between the control and the treated groups. The serum E_2 concentrations of the samples collected from the first day which is the first day oestrus, fifth day which is the first day of cloprostenol sodium ($PGF_{2\alpha}$) to 23rd day

which is the day of next oestrus for all in the both groups and it ranged from 20.08 ± 0.82 to 9.32 ± 1.00 ng/ml (group I) and 14.46 ± 0.52 to 9.22 ± 2.62 ng/ml (group II) while the serum P_4 concentrations of the samples collected from the first day of the experiment which was the day of first oestrus to 23rd day which was the second observed oestrus of all the sows in both groups ranged from 0.78 ± 0.19 to 1.96 ± 0.37 ng/ml (group I) and 1.02 ± 0.15 to 4.70 ± 0.79 ng/ml (group II). Corpus luteum life span following oestrus was also of normal length (21 days) the control and $PGF_{2\alpha}$-treated sows which means that the 24 hourly injections of cloprostenol sodium given daily from Day 5 to Day 10 didn't elicit luteolytic effect on the CL as observable signs of oestrus was seen on average Day 21 of the experiment.

keywords: Oestrus, Synchronization, Cloprostenol Sodium, luteal phase, Repeated luteolysis.

INTRODUCTION

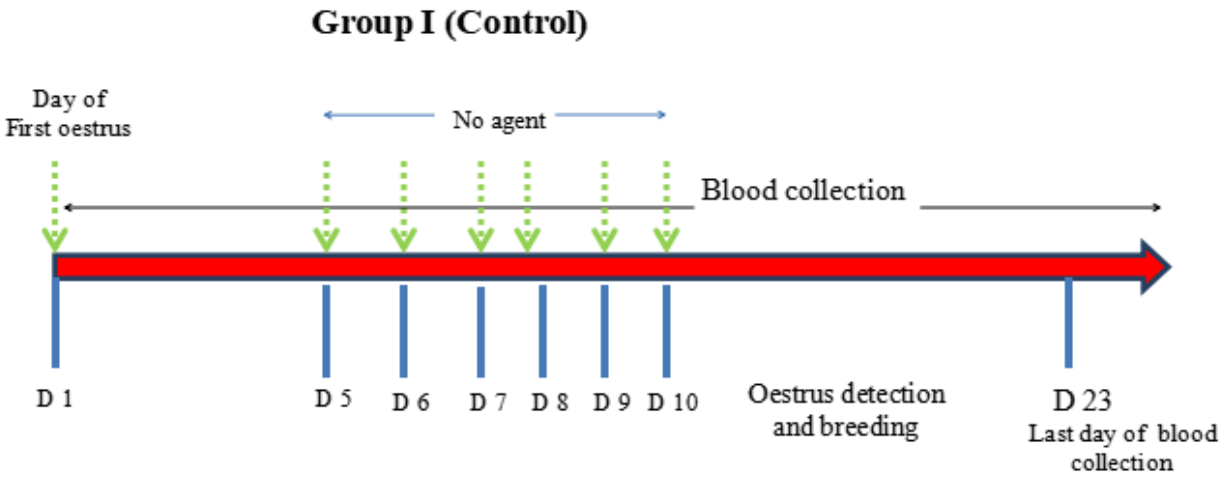
There is much limitation with regard to the availability of methods to alter oestrous cycle in the pig than their counterparts in cattle industry (Estill, 2000) due to its CL refractory nature to $PGF_{2\alpha}$. Because of the relative refractoriness of the porcine *corpus luteum* (CL) to the luteolytic effects of $PGF_{2\alpha}$ during the first 12 days of the cycle. Thus, options for oestrus synchronization in pig production are still very limited, in comparison to options available in the husbandry of other domestic animals, like Jennies (Hassan *et al*., 2020) (De Rensis, and Peter, 1999), sheep (Menchaca *et al*., 2004),and goat (Omotese *et al*., 2016). The insensitivity of sow's CL to the luteolytic effect of $PGF_{2\alpha}$ before day 12 is considered to preclude the use of prostaglandin in oestrus synchronization programmes for swine (Przygrodzka *et al*., 2015). It is however, important that we look for ways of shortening the interoestrous interval to harness the reproductive potential of the pig herd. It is however, important that we look for ways of shortening the interoestrous interval to harness the reproductive potential of the pig herd. Few reports have suggested luteolysis in pigs following multiple injections will result in luteolysis (Estill *et al*., 1993; 1995). However, this repeated administration (Days 5, 6, 7, 8, 9 and 10) of $PGF_{2\alpha}$ 24 hourly during the early phase of the cycle that will help in harnessing most of the benefits of synchronizing oeatrus like increase in return on investment (ROI), saving of labour and batch farrowing which will help in all out all in protocol is swine farm to the best of our knowledge is yet to be evaluated in the Large white X Pietrain in Nigeria. The aim of this study is to determine the effect of repeated injections of cloprostenol sodium during the early luteal phase on luteolytic capacity and hormonal changes of Large white x Pietrain in Otukpo.

MATERIALS AND METHODS

The study was conducted in Department of Theriogenology and Production, Faculty of Veterinary Medicine, ABU Zaria, but the field work was done in Otukpo Swine Research Station of National Animal Production Research Institute (NAPRI), Ahmadu Bello University. Otukpo is located in the southern guinea Savanna, on latitude 7-9° N and longitude 8-10° E and at altitude 490.2 m above sea level. The climate is characterized by two well-defined seasons (BNARDA, 2010).

Twenty (n = 20) apparently healthy sows belonging to the Swine Research Programme of the National Animal Production Research Institute (NAPRI), Otukpo outstation, were used for this study. The sows were 2 - 3 years old weighing from 120 – 150 kg, with an average body condition score of 3.0 using the 0-5 scale from most emaciated to the fattest and had exhibited an oestrous cycle of normal length were selected for this study. The sows were identified by ear notches and fed on the same diet containing 16% crude protein. The ration was formulated to meet the minimum nutrient requirements for breeding sows and boars as recommended by National Research Council (NRC) (1998). The ingredients for the feed were sourced from NAPRI feed store and the ration was mixed in the feed mill in NAPRI, Otukpo station. Water was provided *ad libitum.* Ethical clearance was gotten from ABU committee of animal use and act (Appendix I). The sows were divided into two groups, group I is control while Group II is the treatment group. In group I: No treatment on day 1 (day of detected oestrus) and days 5, 6, 7, 8, 9 and day of first oestrus to day 23 which is the subsequent heat. 10 of the oestrous cycle, they were not given any agent but were monitored for signs of oestrus. 5 ml of blood was collected from day one which is the. The blood was collected every other day that is four times per week till next oestrus which was three weeks and the animals were monitored for natural oestrus and were bred following the second heat (Figure 1). In group II: Each of the five sows in the treatment group received 6 doses of 500 µg of cloprostenol sodium at days 5, 6, 7, 8, 9 and 10 of the oestrous cycle and was monitored for natural oestrus. 5 ml of blood was collected from day one which was the day of first oestrus to day 23 which was the the day of observed second heat. The blood was collected every other day interval that is four times per week till next oestrus which was three weeks and the animals were monitored for natural oestrus and was bred following second heat (Figure 2).

Figure 1: Experimental protocol of sows in group I(control)



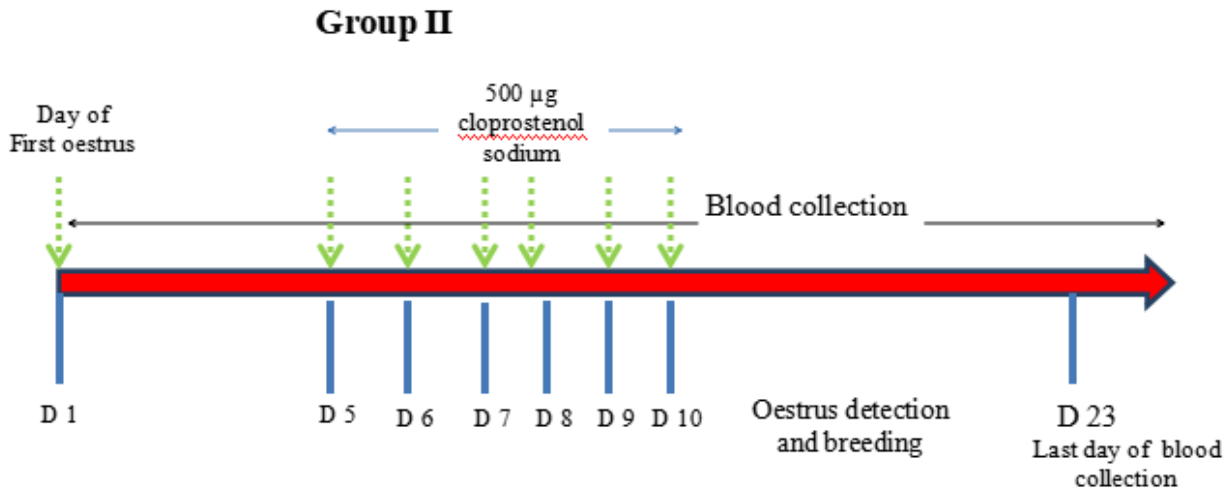


Figure 2: Experimental protocol of sows in group II (showing repeated doses of cloprostenol sodium during the early luteal phase)

Note: D1= day of detected oestrus, D5= fifth day of oestrous cycle and first injection of cloprostenol sodium, D6= sixth day of oestrous cycle, second injection of cloprostenol sodium, D7= seventh day of oestrous cycle, third injection of cloprostenol sodium, D8= eight day of oestrous cycle, fourth injection of cloprostenol sodium, D9= ninth day of oestrous cycle, fifth injection of cloprostenol sodium, D10= tenth day of oestrous cycle, sixth injection of cloprostenol sodium. The sows were observed visually for behavioural oestrus manifestation twice (0700-1000 and 1500-1800 h) daily from commencement of the study for 21 days. The sows were considered to be in oestrus when "stand to be mounted" by females (homosexual mount) or male (heterosexual mount). Parameters were Time to onset of oestrus (TOO), TOO was determined by calculating the time of second oestrus from the selected oestrus sows, Duration of oestrus (DOO), DOO was determined by the time the sows manifested standing oestrus to when the oestrus signs wanes, and litter Size (LS). Five (5) milliliters of blood was collected via the anterior vena cava using a 10-ml hypodermic syringe, fitted with 18 gauge needle from the sows on day 0 (before the commencement of prostglandin treatment) in order to determine the baseline concentration of Oestradiol (E_2) and progesterone (P4), on day 1 (day of first heat), daily from day 5 to 10 just before every 24 hourly cloprostenol sodium injections and every other day until next oestrus to determine concentration of Oestradiol (E2) and progesterone (P4) in order to determine the capability of the corpora lutea to produce P4 (luteolytic capability). Blood samples collected in vacutainers without anticoagulant were quickly transported to the laboratory. Serum samples were separated by centrifugation at 2500 x g for 15 minutes to separate serum from clot. Serum samples were appropriately labelled in vials stored at -20° C until hormone analysis. Serum E_2 and P_4 were determined by using Competitive ELISA kits, the sensitivity of the assay was

0.105 ng/ml. (Within assay precision, coefficients of variation for low, normal and high pooled controlled serum samples were 9.9%, 3.1% and 2.9% respectively.

RESULTS

Time to onset of oestrus (Mean ± S.E.M) was 484.80 ± 13.99hours, and 484.80 ± 15.92 hours in groups I and II respectively. Time to onset of oestrus in both groups was similar. There were no statistical significant ($P \geq 0.05$) differences between the control group and the treatment group (Table 1). The interestrous interval was not significantly ($P \geq 0.05$) different (Table 1). Duration of oestrus (Mean ± S.E.M) was 86.40 ± 5.88 hours and 76.80 ± 4.80 hours in groups I and II respectively. There were not significantly ($P \geq 0.05$) different (Table 1). Total litter size (Mean ± S.E.M) was 10.00 ± 0.84 and 10.20 ± 0.66 in groups I and II respectively. There were not significantly ($P \geq 0.05$) different (Table 1). Total born alive (Mean ± S.E.M) was 10.00 ± 0.84 and 10.00 ± 0.55 in groups I and II respectively. There were not significantly ($P \geq 0.05$) different (Table 1). Average body weight (Mean ± S.E.M) was 1.26 ± 0.09 kg and 1.06 ± 0.05 kg in groups groups I and II respectively. There were not significantly ($P \geq 0.05$) different (Table 1).

Table 1: Fertility paramaters (onset and duration of oestrus, litter size and birth weight) of control group and group treated with repeated administration of cloprostenol sodium during the early luteal phase.

PARAMETERS	GROUP I	GROUP II	P-Value	SIGNIFICANCE
TOO (hrs)	484.80 ± 13.99	484.80 ± 15.92	1.000	NS
DOO (hrs)	86.40 ± 5.88	76.80 ± 4.80	0.178	NS
TLS (n)	10.00 ± 0.84	10.20 ± 0.66	0.883	NS
TLB (n)	10.00 ± 0.84	10.00 ± 0.55	1.000	NS
Av BW (kg)	1.26 ± 0.09	1.06 ± 0.05	0.154	NS

No statistical ($P \geq 0.05$) significance

NOTE: TOO in control is the time of onset of oestrusis the interval between two oestrus, while in treated group, time to onset of oestrus (TOO) (interval between the day of last injection of prostaglandins and onset of oestrus).

Key:

TOO = Time to onset of oestrus following first and second $PGF_{2\alpha}$ injection
DOO = Duration of oestrus
TLS= Total litter size
TBA = Total born alive
Av BW = Average body weight
NS = Not significant
hrs = hours
n = number
Kg = Kilogram

The mean concentrations of both hormones (estradiol-17β and progesterone) in the sera samples collected were evaluated for each

day in the two groups I and II. E_2 concentrations of the samples collected from the first day which was the first day oestrus, fifth day which was the first day of cloprostenol sodium ($PGF_{2\alpha}$) to 23rd day which was the day of next oestrus for all in the both groups and it ranged from 20.08 ± 0.82 to 9.32 ± 1.00 ng/ml (group I) and 14.46 ± 0.52 to 9.22 ± 2.62 ng/ml (group II) while the serum P_4 concentrations of the samples collected from the first day of the experiment which was the oestrus day to 23rd day which was the next heat of all the sows in the both groups and it ranged from 0.78 ± 0.19 to 1.96 ± 0.37 ng/ml (group I) and 1.02 ± 0.15 to 4.70 ± 0.79 ng/ml (Figure 3).

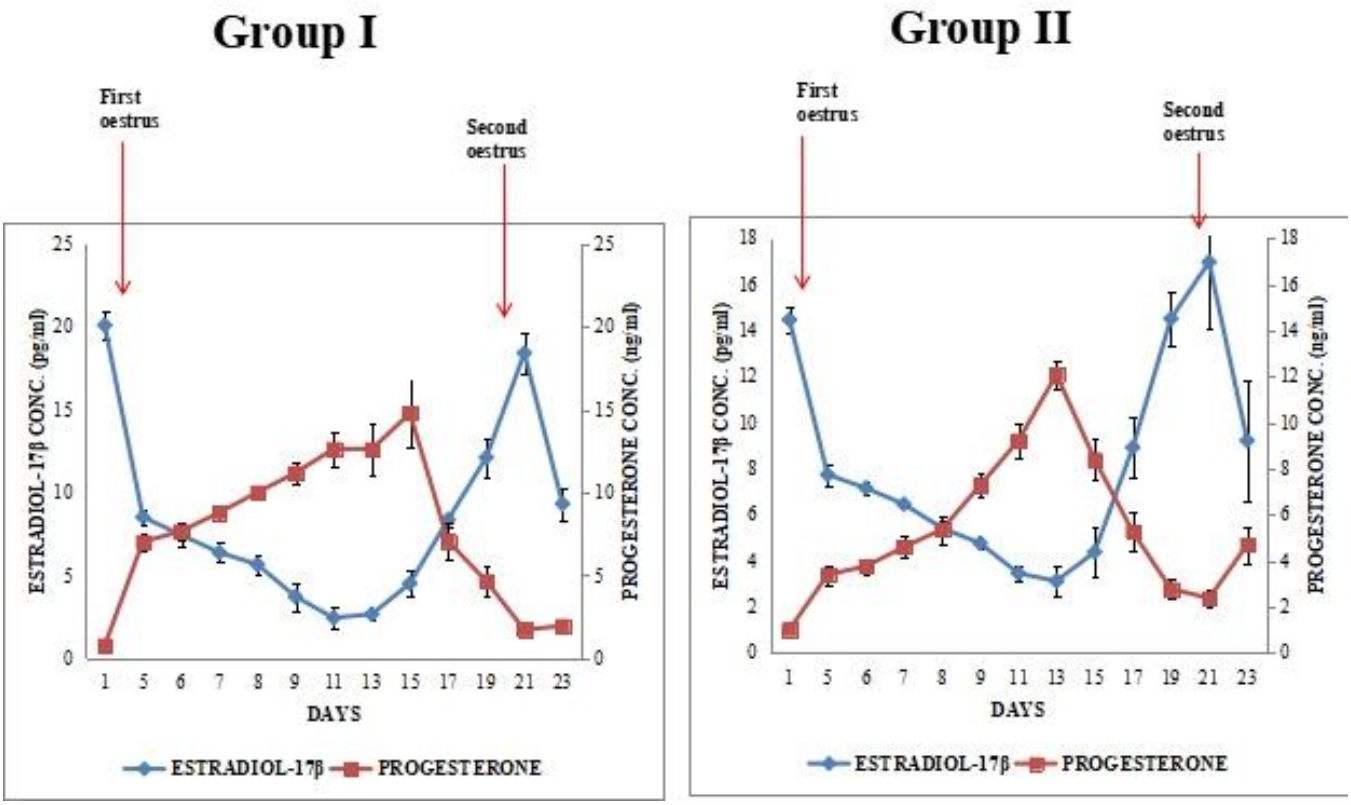


Figure 3: Mean (± SEM) serum estradiol-17β and progesterone concentration in sows in Control and treatment group.

DISCUSSION

The results of this study have shown that repeated injection of cloprotenol sodium (Synchromate®) at 24 hour interval during the early luteal phase is not luteolytic in porcine 'CL this' contradicts the reports of Estill *et al*., 1993 and 1995, the reason for this disparity could be the time interval of injections of the $PGF_{2\alpha}$. Time to onset of oestrus observed in this study was similar between the control (484.80 ±13.99 hours) and the treated (484.80 ± 15.92 hours) groups which is in contrast to that reported by Estill *et al*. (1993) who reported mean interestrous interval in $PGF_{2\alpha}$-treated gilts was reduced to 13.3 ± 0.5 days compared with 19.8 ± 0.6 days for control gilts using 12.5 mg $PGF_{2\alpha}$ (dinoprost tromethamine) i.m. every 12 hour. The mean oestrous length in both groups was similar and was longer than that by Estill *et al*. (1993) who reported the mean oestrous cycle length for sows to be 19 days and 19.7 days for both treated with 12.5 mg $PGF_{2\alpha}$ and untreated sows respectively. Duration of oestrus was similar for groups, 86.40 ± 5.88 hours (control) and 76.80 ± 4.80 hours (treated). The duration of oestrus for the control group is similar with that reported by Oke-Egbodo *et al* (2017) (96.00 ± 3.79 h and 86.50 ± 5.85 h) while the treated group is shorter though not significantly different. This could be due to the continous lumsum effect of the repeated injections of cloprostenol sodium after day 10 of the oestrous cycle. No significant differences were observed between the litter size of treated group and that of the control, this corroborates the reports of Kirkwood *et al.* (2007) who reported no effect of cloprostenol sodium (175 and 525 µg) on the litter size. The total born alive observed in this study was generally higher than that reported by Kirkwood, (1997) (9.7 ± 0.3) using GnRH, while the average body weight in this our work was not significantly different between the control and the treated groups which indicated that synchronizing the animals does not affect the piglet weight at birth. In both control and the treated groups the mean E_2 concentration decreased from 20.08 ± 0.82 and 14.46 ± 0.52 pg/ml respectively on day 1 (the day of first oestrus) to 2.44 ± 0.66 pg/ml on day 11 and 3.12 ± 0.65 pg/ml on day 13 respectively. This means that the 24 hourly injections of 500 ug of cloprostenol sodium from Day 5 to 10 does not have effect on the CL as there was consequent increase in the baseline P_4 (0.78 ± 0.19 and 1.02 ± 0.15 ng/ml) to peak 14.78 ± 2.05 and 12.08 ± 0.57 ng/ml at day 15 and 13 for groups I and II respectively. This means that luteolysis was not reported and this work does not agree with the works of Estill *et al*. (1993, 1995) who demonstrated prematured luteolysis following 12 hourly injections of 12.5 mg dinoprostthrometamine during the early luteal phase. This work also agrees with earlier reports of Oke-Egbodo *et al*., 2018 on the refractoriness of CL to $PGF_{2\alpha}$ prior to 13 days of the oestrous cycle. Other similar studies have shown that multiple injection of $PGF_{2\alpha}$ have little or no effects on CL (Diehl and Day, 1974; Hallford *et al.,* 1975; Guthrie and Polge, 1976; Connor *et al*., 1976; Kryzmowski *et al*., 1978; Berghorn *et al*., 1989). The elevated concentrations of serum oestradiol prior to the onset of oestrus in both control and $PGF_{2\alpha}$ treated sows indicated that follicular maturation and ovulation occurred normally. Corpus luteum life span following oestrus was also of normal length (21 days) in both the control and $PGF_{2\alpha}$-treated sows which means that the cloprostenol sodium

given daily from Day 5 to Day 10 didn't elicit luteolytic effect on the CL as observable signs of oestrus was seen on Day 21 of the experiment meaning that the cloprostenol sodium 24 hourly was not enough to cause the up-regulation of its own receptor for the $PGF_{2\alpha}$ receptor concentration to increase and render the corpus luteum sensitive to $PGF_{2\alpha}$ (Estill *et al*., 1993, de Rensis *et al*., 2012; Oke-Egbodo *et al*., 2018). Based on the findings it is concluded that repeated doses of cloprostenol sodium 24 hourly and at the tested dose during the early luteal phase is not efficacious in oestrus synchronization in Large white x pietrain breed of sows and repeated doses of 500 ug cloprostenol sodium 24 hourly were not luteolytic during the early (Days 5, 6, 7, 8, 9 and 10) luteal phase of the oestrous cycle in sows. Further studies on other frequencies of multiple injections of cloprostenol sodium should be given trial to see if there will be effect on the CL to cause premature luteolysis. It is recommended that longer-acting $PGF_{2\alpha}$ analogues may allow this treatment regimen to be refined further hence should be tried and use of more animals for future studies is recommended in order to have a high pool of data for analysis and possible better results.

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