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Pattern of induced estrus during superovulatory programme in Kangayam donor cows

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Abstract
The objective of the present study is to report the pattern of induced estrus before the superovulatory treatment in Kangayam donor cows. A total of 16 Kangayam donor cows were selected for this study. The animals were treated with two doses of 500 µg $PGF_2\alpha$ intramuscularly at an interval of 11 days. At 24 hours after the administration of 2nd dose of $PGF_2\alpha$, all the Kangayam donor cows were observed for estrus response, onset of estrum, sign of induced estrus, duration of induced estrus, fern pattern and intensity of estrus. The onset of estrus was 61.00±1.89 hours, duration of estrus was 18.25±1.4 hours and the intensities of estrus were 62.5%, 25% and 12.5% as intense, intermediate and weak respectively in Kangayam donor cows.

Keywords: Kangayam breed, donor cows, estrus pattern, induced estrus

Introduction
There are 50 registered indigenous cattle breeds in India. Kangayam cow is a pride indigenous breed of Tamil Nadu. Embryo transfer technology (ETT) is a biotechnological tool by which embryos are collected from a superior donor female and are transferred to recipient females, which serve as surrogate mothers for the remainder of pregnancy. The basic step for ETT is synchronization of estrus in donor cows after selection.

The estrus synchronization is a hormonal regulation of estrous cycle at a time in a group of animals with timed artificial insemination (AI). It allows decrease in unproductive periods by controlling the postpartum sub estrus or anestrus. Synchronization reduces the number of days spent visually appearing estrus; it also increases the accuracy of heat detection (Ratnaparkhi *et al.*, 2020) [7]. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has so far been the most frequently used hormone for estrus synchronization in cows (Patterson *et al.*, 2003) [6]. $PGF_{2\alpha}$ controls life span of the corpus luteum, but cannot alter the course of follicular waves. A single treatment with the recommended dose of PG $F_{2\alpha}$ in the first 4 days of the cycle is unable to induce luteolysis (Rowson *et al.*, 1972; Momont and Sequin., 1984 and Beal *et al.*, 1988) [8, 4, 2]. There is no report of pattern of synchronized/induced estrus in Kangayam donor cows. This study is the first attempt in documenting the pattern of synchronized/induced estrum during superovulatory programme in Kangayam donor cows. Therefore, double PG injections at an interval of 11 days were tried in Kangayam donors to study the efficacy of estrus induction to facilitate the superovulatory programme.

Materials and Methods
Source of Experimental Animals
An experiment was conducted in Kangayam cows which are maintained for embryo transfer programme in the Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Namakkal and also reared by the progressive farmers in different villages of Namakkal and Erode Districts of Tamil Nadu.

Selection of Experimental Animals
A total of 16 numbers of healthy, pluriparous Kangayam cows aged between 2nd and 5th parity at ≥ 60 days postpartum were selected based on the phenotypic characters with reference to the Kangayam breed and without any palpable abnormalities of the genital tract.

These donor cows were tested for infectious diseases such as brucellosis, tuberculosis and Johne's disease and were found to be negative. The body condition score of selected donors were between 3 and 4 and their milk production was above 6 litres /day/cow.

Synchronization of estrus

All the selected Kangayam donor cows (n=16) were treated with two doses of 500 µg $PGF_2\alpha$ (Cloprostenol, PG Estro™, Hester Biosciences limited, India) through intramuscularly at an interval of 11 days. At 24 hours after the administration of 2nd dose of $PGF_2\alpha$, all the donor cows were observed for estrus signs.

Pattern of induced estrus in donor cows

The experimental donor cows were closely observed for estrus signs at 24 hours after second $PGF_2\alpha$ in the both groups**.**

1. Estrus response

The donor cows were closely observed for the estrus signs particularly 24 hours after 2nd $PGF_2\alpha$ injection (Day 11 morning) in both the groups. Estrus response in percentage was calculated as number of cows expressed estrus divided by the number of animals treated in each group and multiplied by 100.

2. Onset of estrus

Onset of estrus was calculated in terms of hours, from the time of 2nd $PGF_2\alpha$ administration to the time of first appearance of estrus signs in both the groups.

3. Signs of induced estrus

After the onset of induced estrus, the donor cows were carefully observed for identification of estrus signs (30 minutes close observation 3 times in a day).

4. Duration of induced estrus

Duration of induced estrum was estimated in hours from the appearance of first estrus signs to disappearance of estrus signs in both the groups.

5. Fern pattern in induced estrus

Cervical mucus samples were collected from all cows two times at 12 hours interval after the onset of estrus. The vulva of the cow was washed with disinfectant solution, rinsed with water and then dried. A plastic flexible sheath was used for the collection of cervical mucus samples. A 10 ml plastic syringe was attached to the external end of the sheath, the sample was collected from the external os of the cervix by transrectal method in each donor and the mucus was spread on a clean microscope slide, labelled for identification and dried at low temperature over an alcohol burner. After that, slides were subjected to microscopical examination for grading the fern pattern of induced estrus in donor cows.

6. Intensity of induced estrus

The intensity of estrus was measured based on the score card described by Selvaraju (2005) [10] in repeat breeders with slight modifications. The modifications include instead of mounting, standing to be mounted and posture in the score card, behavioral changes as bellowing/vocalization, reduction in the feed intake, and tactile sensation in genital tract were included. The detail of the score card is given below.

Table 1: Score card

S. No	Parameters	Points		
1	**Behavioral changes**			5
	Restlessness	2		
	Bellowing	1		
	Reduction in feed intake	1		
	Tactile sensation in genital region	1		
2	**Physiological changes**			5
	Vulval oedema		2	
	Highly oedematous Edematous Not oedematous	2 1 0		
	Urination		1	
	Genital discharge		2	
	Copious Moderate volume Scanty	2 1 0		
3	**Gynaecological observation**			5
	Fern pattern		2	
	Typical fern pattern Atypical fern pattern	2 1		
	Rectal examination		2	
	Cervical relaxation	2		
	Uterine tonicity		2	
	High Moderate Flaccid	2 1 0		
	Total	15		
.	Based on the above score card, the intensity of estrus was classified as follows. Intense: 10 - 15 points Intermediate: 05 - 10 points Weak: 00 - 05 points			

Results and Discussion

In the present study, all the 16 donor cows exhibited estrus following the second PG injection. Hence, there was 100 per cent estrus response following double PG schedule for synchronization of estrus in Kangayam cows. Injection of $PGF_{2\alpha}$ on day 10 following natural estrus in repeat breeder cows resulted in 100 per cent estrus response (Selvaraju *et al.*, 2008 and Selvaraju *et al.*, 2010) [11, 12]. Similarly, Selvaraju *et al.*, (2011) [13] obtained 100 per cent estrus response following norgestomet ear implant treatment in cows. Velladurai *et al*., (2014) [14] recorded 100 per cent estrus response in cows treated with ovsynch protocol. On contrary, single $PGF_{2\alpha}$ treatment on day 5 of the estrus cycle caused 41 % estrus response in heifers and 0% in lactating cows. But on day 7, single injection of $PGF_{2\alpha}$ yielded 90 % estrus response in lactating cows. Therefore physiological status of the cow at the time of PG injection influenced the estrus response (Nascimento *et al.*, 2014) [5]. The double PG injections at an interval of 11 days in this study assured the presence of responding corpus luteum at the time of second PG injection and might have resulted in complete luteolysis to exhibit at 100% estrus response as described by Ratnaparkhi *et al*., (2020) [7].

The mean onset of estrum after second $PGF_{2\alpha}$ in double PG schedule in Kangayam cows was 61.00±1.89 hours. Ratnaparkhi *et al.*, (2020) [7] recorded 56.40±2.20 hours as average time required for onset of estrus in crossbred dairy cows treated with double PG. Selvaraju *et al.*, (2008) [11] observed the onset of estrum following single PG injection on day 10 of the cycle in repeat breeder cows as 59.38±0.81 hours. Velladurai *et al.*, (2015a) [15] treated the normally calved cows with ovsynch protocol and found the onset of estrus as 47.97±2.65 hours. Sahatpure and Patil (2008) [11] recorded 54.40±2.60 and 55.58±3.28 hours average time interval for onset of induced estrus in non-descript crossbred cows with double dose of $PGF_{2\alpha}$ injection at 11 days apart, respectively. Similarly, Ahlawat *et al.*, (2015) [1] found the mean (±) SE time interval to onset of estrum with double doses of $PGF_{2\alpha}$ at 11 days apart as 56.86±1.96 hours in cows.

The duration of synchronized estrus following double PF injection in Kangayam cow (Table. 2) was 18.25±1.4 hours. Ratnaparkhi *et al.*, (2020) [7] recorded the mean duration of estrus in ovsynch and double $PGF_{2\alpha}$ protocol as 22.80±0.44 and 23.80±55 hours respectively in crossbred cows. Velladurai *et al.*, (2015b) [16] treated the RFM affected cows with ovsynch and ovsynch+mineral mixture and reported 29.38±0.74 and 28.35±0.63 hours as duration of induced estrus and they reported mineral supplementation did not affect the duration of estrus in cows. Selvaraju *et al., (*2005) [10] studied the effect of estrus synchronization aspects on estrus pattern in repeat breeder cows and estimated the duration of estrus a 28.50±0.56 hours. However, in the present study the duration of estrus was less than previous reports. It might be due to the breed variation in relation to Kangayam breed undertaken in the study.

The intensity of estrus was measured based on the score card. Similar score card was used in other studies (Selvaraju *et al.*, 2005; Velladurai *et al.*, 2014 and Ratnaparkhi *et al.*, 2020) [10, 14, 7]. The percentages of intense, intermediate and weak intensity obtained in the study were 62.5, 25 and 12.5% respectively. Similar classification of estrus intensity was followed by Ratnaparkhi *et al*., (2020) [7] in crossbred dairy cows. They obtained 40, 40 and 20 percent and 50, 30 and 20 percent intense, intermediate and weak estrus intensity in ovsynch treated and double PG injected crossbred cows, respectively. The percentages of very good and good estrus intensity were 81.25 and 18.75, 75.00 and 25; 87.50 and 12.50 and 25.00 and 75.00% in norgestomet, norgestomet+PG, PG and control treated repeat breeder cows (Selvaraju *et al.*, 2005) [10]. In the present study 12.5% weak estrus intensity was observed following PG injection in Kangayam cows. Ratnaparkhi *et al.*, (2020) [7] found higher estrus intensity in double PG protocol compared to ovsynch protocol. Similar to the present study weak estrus intensity of 20% was recorded by Deshmukh *et al.*, (2015) [3]. In the current experiment, intense (62.5%), intermediate (25%) and weak (12.5%) estrus intensity were reported. Hence, double PG injection did not affect the estrus intensity in Kangayam cows. Thus from this study, it was concluded that estrus response, onset of estrus, duration of estrus and intensity of estrus in Kangayam cows were not affected with double PG injection in donor cows and this schedule can be used for induction of estrum in Kangayam donor cows.

Table. 2: Pattern of induced estrus in kangayam donor cows

Synchronized estrus				
Onset of estrus (hrs)	Duration of estrus (hrs)	Intensity of estrus		
		Intense	Intermediate	Weak
61.00±1.89	18.25±1.4	62.5%	25%	12.5%

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