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Comparative Study on Effect of Different Estrus Synchronization Protocols on the Pattern of Estrus, Conception Rate and Serum Hormonal Profile in Indigenous Kangayam Cows

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ABSTRACT

Background: The indiscriminate crossbreeding is the major cause for losses as well as for risk to existing breeds. Kangayam, a pride indigenous cattle breed of Tamil Nadu, also badly affected due to this unplanned indiscriminate breeding. Urgent and immediate action is required to protect this traditional breed. Estrus synchronization would be a best tool to improve the fertility rate and increase the population of this breed under field condition.

Methods: Fifty Kangayam cows selected at farmer's field were divided into Group I, II, III, IV (Treatment groups) and Group V (Control group). Estrus synchronization with single $PGF_2\alpha$, double $PGF_2\alpha$, CIDR+$PGF_2\alpha$ and ovsynch protocols were used in Group I, II, III and IV cows, respectively and inseminated during induced estrus. Group V cows were inseminated during observed estrus. The pattern of estrus, fertility rate and serum hormonal profiles on different days of treatment were studied.

Result: The conception rates were higher in double $PGF_2\alpha$ (80 per cent) and ovsynch protocol (80%) followed by CIDR+ $PGF_2\alpha$ (70%). The progesterone (ng/ml) concentration was significantly ($P \leq 0.05$) higher during estrus induction with $PGF_2\alpha$ in treatment groups than during selection and insemination. The serum estradiol-17β concentration was significantly ($P \leq 0.05$) higher during insemination (24.21±1.74 to 25.57±0.80 pg/ml) than during $PGF_2\alpha$ injection (12.81±0.25 to 13.68±0.30 pg/ml). The study concluded that higher conception rate in indigenous Kangayam cows can be obtained with synchronization using Double $PGF_2\alpha$ and Ovsynch protocol followed by CIDR+$PGF_2\alpha$ protocol.

Key words: CIDR, Conception rate, Estrus synchronization, GnRH, Indigenous breed, Kangayam cow, Ovsynch, $PGF_2\alpha$.

INTRODUCTION

Among various indigenous breeds of Tamil Nadu, Kangayam is an excellent draught breed. The average lactational milk yield of Kangayam cows was 642 to 921 kgs but some superior Kangayam cows produced 2839 kgs. The age at puberty ranged from 29.49 to 33.08 months and the age at first calving was 38 to 45 months (Panneerselvam and Kandasamy, 2008). The data's indicate the potentiality of the breed. As per Estimated Livestock Population Breed-wise based on Breed Survey-2013, the pure Kangayam population and graded Kangayam animal population was 80,620 and 1,12,825, respectively. The drastic changes in the livestock industry have made strong focus towards high yielding animals (Jaisuriyan *et al*., 2021) and the Kangayam breed was also affected due to this. If the trend continues, the population may drastically come down and may reach the endangered state soon. Hence there is an urgent need for retain and propagate superior Kangayam animals to increase its population. For this the modern reproductive biotechnologies may be used.

Synchronization of estrus is a reproductive bio-technological tool which indicates the manipulation of the estrous cycle or induction of estrus to bring a large number or group of female animals into estrus at the same time. Estrus synchronization provides higher economic returns to farmers by improving the production efficiency in animals (Pursley *et al*., 1995). There are several synchronization protocols are available for synchronizing estrus among female animals. Traditional protocols are formulated to mimic or control the corpus luteum on the ovary. The recent protocols are designed to control ovulation or to control the follicular waves. After synchronization, fertility in farm animals is expected towards higher side because of the timely breeding carried out. Further this technique establishes sustainability in the dairy industry (Nakrani *et al*., 2014) and thereby improves hopefulness among dairy farmers. The estrus synchronization protocols yielded better results in exotic/crossbred animals but studies on indigenous animals are scarce. Hence the present study was carried

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out to find out the efficiency of various estrous synchronization protocols on estrus induction and improving the fertility rate in indigenous Kangayam cows under field conditions.

MATERIALS AND METHODS

The present study was conducted in the Kangayam Cattle Research Station, Tamil Nadu Veterinary and Animal Sciences University, Baguthamplayam, Sathyamangalam during December 2019 to January 2021.

A total number of 50 pluriparous, healthy Kangayam cows between 2nd and 5th parity were selected at farmer's field in the breeding tract during estrus for the study. The animals having 2.5 and above BCS (body condition scoring) were subjected to a thorough gynaeco-clinical examination and those cows with no palpable abnormalities of the genital tract and negative for white side test with uterine discharge were utilized for the experiment. The selected cows were divided into five experimental groups *viz.,* Group I, II, III, IV (Treatment groups) and Group V (Control group) and supplemented with TANUVAS mineral mixture for 25 days. The cows were subjected to one of the four estrus synchronization protocols *viz*., Single $PGF_2\alpha$ Double $PGF_2\alpha$, CIDR+ $PGF_2\alpha$ and Ovsynch and were artificially inseminated with good quality frozen thawed Kangayam semen. No treatment was given for control group animals.

Group I (Single $PGF_2\alpha$, protocol)

The animals in this group were examined per rectum for the confirmation of matured corpus luteum and received single injection of 500 µg synthetic $PGF_2\alpha$,intramuscularly and inseminated on exhibition of estrus.

Group II (Double $PGF_2\alpha$ protocol)

The cows were given two doses of 500 µg synthetic $PGF_2\alpha$ intramuscularly at an interval of 11 days and inseminated on exhibition of estrus.

Group III (CIDR+$PGF_2\alpha$ protocol)

The experimental animals were inserted with CIDR intravaginal device with special applicator. An intramuscular injection of 500 mg $PGF_2\alpha$, was given on day 6, CIDR removed on day 7 and inseminated on exhibition of estrus.

Group IV (Ovsynch protocol)

The animals were administered with intramuscular injections of 10 µg of GnRH on the day of start of synchronization of ovulation (day 0) followed by 500 µg synthetic $PGF_2\alpha$ seven days later (day 7) and another 10 µg of GnRH 48 hours after the $PGF_2\alpha$(day 9) and timed artificial insemination (TAI) at 16-18 hours after the second GnRH injection (day 10).

Group V (Control)

The control group cows were supplemented with mineral mixture and inseminated during first estrus after supplementation.

Parameters studied

Pattern of induced estrus in experimental cows

The experimental cows were closely observed for estrus signs 24 hours after estrus induction. The pattern of induced estrus *viz.* estrus response, onset of induced estrus, duration of induced estrus and intensity of induced estrus was studied as explained by Periyannan *et al*. (2021). In the control group, the duration and intensity of estrum were studied in natural estrus before insemination.

Conception rate

All the cows were monitored regularly following insemination at induced estrus in groups I to IV and at observed estrus in group V for non-returning of estrum. The cows which have not expressed heat signs were confirmed for pregnancy by rectal palpation at 60 days post insemination. The conception rate was expressed in percentage as first service, second service and overall conception rate in the experimental and control animals.

Hormone assay

In experimental animals, blood collection was done during (i) selection of Kangayam cows (ii) on the day of $PGF_2\alpha$, injection in group I, III and IV/on second $PGF_2\alpha$ injection in group II (iii) on the day of AI and (iv) 10 days following AI. In group V animals, blood was collected at the (i) time of selection (ii) AI and (iii) 10 days following AI. The serum was separated and stored at -20°C until analysis. The serum samples were analysed for progesterone and estrogen by radioimmunoassay (RIA) technique.

Statistical analysis

The data on pattern of induced estrus and conception rates of different estrus synchronization protocols were tested by using chi square test on arc sin transformed data. The serum progesterone and estradiol-17β profiles were analyzed statistically through analysis of variance using online SAS software version 20.00.

RESULTS AND DISCUSSION

Estrus synchronization is the most important and widely applicable reproductive biotechnological tool available for cattle. If the protocol is used properly, it would be most useful to propagate the indigenous animal's population. In the present study four different estrus synchronization protocols have been used to assess their effect on the fertility rate in indigenous Kangayam cows.

In the present study, all the experimental animals of groups I to IV exhibited estrus (100 per cent) following estrus synchronization (Table 1). The result of this study was in accordance with the observations of Saini *et al*. (2016) in buffaloes and Periyannan *et al*. (2021) in Kangayam cows. Devipriya *et al*. (2020) recorded a slightly lower response of 90 and 80 per cent estrus induction rate in Kangayam and crossbred Jersey cows after synchronization with progesterone sponges. Manokaran *et al*. (2019) obtained 100 per cent estrus response in repeat breeder cows following ovsynch protocol. Nasicimento *et al*. (2014) used single $PGF_2\alpha$ treatment on day 5 of the estrus cycle and obtained 41 and 0 per cent estrus response in heifers and

Table 1: Pattern of estrus and conception rate in Kangayam cows in response to different estrus synchronization protocols.

Response details		Single $PGF_2\alpha$ (Group I)	Double $PGF_2\alpha$ (Group II)	CIDR+$PGF_2\alpha$ (Group III)	Ovsynch (Group IV)	Control (Group V)
No. of cows studied		10	10	10	10	10
No. of cows expressed estrus (%)		10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Onset of induced estrus (Mean ±SE) (hours)		61.90^{y}±2.37	52.90^{x}±2.20	59.40^{y}±2.73	53.30^{x}±2.36	-
Duration of induced estrus (Mean ±SE) (hours)		17.00^{y}±1.17	16.60^{y}±0.74	17.20^{y}±0.95	16.30^{y}±0.74	14.00^{x}±0.82
Intensity of estrus	Intense (%)	2 (20.00)	3 (30.00)	2 (20.00)	4 (40.00)	2 (20.00)
	Intermediate (%)	5 (50.00)	5 (50.00)	6 (60.00)	4 (40.00)	4 (40.00)
	Weak (%)	3 (30.00)	2 (20.00)	2 (20.00)	2 (20.00)	4 (40.00)
First service conception rate (%)		4 (40)	6 (60)	5 (50)	6 (60)	2 (20)
Second service conception rate (%)		2 (33.33)	2 (50)	2 (40)	2 (50)	2 (25)
Overall conception rate (%)		6 (60)	8 (80)	7 (70)	8 (80)	4 (40)

Means bearing different superscripts (x, y) between column in the same row differ significantly (P≤0.05).

lactating cows. But $PGF_2\alpha$ injection on day 7 yielded 90 per cent estrus response in lactating cows. Based on the results they concluded that physiological status of the cow at the time of $PGF_2\alpha$ injection influenced the estrus response. The 100 per cent estrus response following estrus synchronization protocols in groups I to IV might be due to the fact that these cows were in diestrum stage at the time $PGF_2\alpha$ injection and might have resulted in complete luteolysis (Manokaran *et al*., 2019) which was also indicated by the blood progesterone concentration analyzed in the study.

The mean time taken for the onset of estrus following estrus synchronization in Kangayam cows in group I, II, III and IV was 61.90±2.37, 52.90±2.20, 59.40±2.73 and 53.30±2.36 hours, respectively (Table 1). Statistically, the mean onset of estrus in group I and III showed significant difference (Pd≤0.05) with group II and IV. Ratnaparkhi *et al*. (2020) and Periyannan *et al*. (2021) recorded 56.40±2.20 and 61.00±1.86 hours for the onset of estrus in crossbred and Kangayam cows following double $PGF_2\alpha$ injection. Devipriya *et al*. (2020) observed 62.00±6.95 and 38.00±5.66 hours for the onset of estrus in Kangayam and Jersey crossbred cows following synchronization with progesterone sponges. Dhami *et al*. (2015) reported 58.27±1.93, 64.13±1.33 and 54.00±1.17 hours as the time required for the onset of estrus after synchronization with CIDR, ovsynch and norgestomet ear implant protocols in anestrus crossbred cows. Manokaran *et al*. (2019) recorded 46.70±1.27 and 44.70±1.11 hours during high breeding season and 52.80±1.10 and 53.20±0.92 hours during low breeding season for the onset of estrus in repeat breeder cows following treatment with ovsynch and ovsynch+vitamin A protocol, respectively. In the present study, the mean duration of induced estrus was 17.00±1.17, 16.60±0.74, 17.20±0.95 and 16.30±0.74 hours in group I to IV, respectively. The mean duration of estrum was 14.00±0.82 hours in group V. The mean duration of induced estrus in groups I, II, III and IV had no significant difference (Pe≥0.05) among them whereas the group V had significant difference (Pd≤0.05) with other groups. In accordance with this study, Periyannan *et al*. (2021) reported 18.25±1.40 hours as mean duration of induced estrus after double $PGF_2\alpha$ injection and Devipriya *et al*. (2020) reported 18.00±0.26 hours after induction with intravaginal progesterone sponges in Kangayam cows. Ratnaparkhi *et al*. (2020) recorded 22.80±0.44 and 23.80±55 hours as mean duration of estrus following ovsynch and double $PGF_2\alpha$ protocol, respectively in crossbred cows which was higher than the observations of this study. The duration of induced estrus recorded in the present study was less than the previous records in crossbred animals which might be due to the breed variation as the Kangayam breed was undertaken in the study.

The percentages of intense, intermediate and weak estrus intensity obtained in the study were 20.00, 50.00 and 30.00 in group I, 30.00, 50.00 and 20.00 in group II, 20.00, 60.00 and 20.00 in group III, 40.00, 40.00 and 20.00 in group IV and 20.00, 40.00 and 40.00 in group V, respectively. In the present study, the weak estrus intensity was lower in induced estrus than natural estrus. Similar to this study, Manokaran *et al*. (2019) recorded more intense and intermediate estrus intensity in repeat breeder cows synchronized with ovsynch protocol during high and low breeding season and concluded that prompt follicular development following synchronization might be the reason for higher estrus expression rates in experimental cows. In the present study also it was confirmed by the serum estradiol concentration obtained during induced estrus of experimental cows which was higher than the natural estrus in control cows.

The first service, second service and overall conception rate after synchronization was studied and presented in Table 1. The results of this study showed that a significantly improved conception rate following estrus synchronization in Kangayam cows. Among the various groups, the conception rates were higher in double $PGF_2\alpha$(80 per cent) and ovsynch

protocol (80 per cent) followed by CIDR+ $PGF_2\alpha$ (70 per cent). In corroboration to this study, Yeshimebet *et al*. (2017) obtained higher conception rate following double $PGF_2\alpha$ protocol (63.10 per cent) than single $PGF_2\alpha$ protocol (55.80 per cent) in dairy cows. When single $PGF_2\alpha$ is given, the hormone controls the life span of the corpus luteum, but could not be able to alter the course of follicular waves and hence the conception rate varies. In double $PGF_2\alpha$ protocol, the second $PGF_2\alpha$ injected 11 days after first injection control the follicular waves and causes tight synchrony of estrus and thereby results in higher conception rate (Islam *et al*., 2011). Dhami *et al*. (2015) recorded 60, 25 and 33.33 per cent in CIDR+ $PGF_2\alpha$ group and 50, 40 and 33.33 in ovsynch group as first, second and third service conception rates, respectively in anestrus crossbred cows with overall conception rate of 80 per cent in both the groups, respectively. Whereas Naikoo *et al*. (2016) obtained a lower conception rate of 16.66, 00.00 and 20.00 per cent in ovsynch group and 33.33, 25.00 and 00.00 per cent in CIDR group as first, second and third service conception rates, respectively in postpartum anestrus Kankrej cows and overall conception rate was 33.33 and 50 per cent in both the groups, respectively. Ratnaparkhi *et al*. (2020) obtained 50 and 40 per cent conception rate following ovsynch and double $PGF_2\alpha$ treatment in crossbred dairy cows. The CIDR effectively synchronizes the estrus because it prevents the animals being in early stage of cycle and non-responsive to $PGF_2\alpha$ induced regression of corpus luteum (Islam, 2011). The combination of CIDR+ $PGF_2\alpha$ treatment could increase the synchronization rate by approximately 30 per cent and pregnancy rate by approximately 20 per cent than single $PGF_2\alpha$ treatment in both anestrus and cyclic dairy cows (Lucy *et al*., 2001). In ovsynch, the first GnRH alters the follicular growth by inducing ovulation in dominant follicle to form new or additional corpus luteum. From the new group of follicle emerged after the first GnRH injection, the dominant follicle ovulate after $PGF_2\alpha$ injection (Pursley *et al*., 1995). This protocol causes tight synchronization among the females and higher conception rate (Islam, 2011). The results proved that synchronization protocols could be used in indigenous Kangayam animals to increase the conception rate and thereby the population can be increased.

The mean progesterone (P_4) concentration was analyzed on the day of selection, on the day of $PGF_2\alpha$ injection (estrus induction), on the day of artificial insemination and 10 days after insemination and is depicted in the Table 2. The data show that the mean P_4 concentrations were towards basal level during selection as the animals were selected during estrum. The concentration subsequently rose significantly ($P\leq0.05$) during estrus induction (2.39±0.32, 3.35±0.16, 3.14±0.16 and 3.31±0.16 ng/ml) in all the groups particularly in double $PGF_2\alpha$ CIDR+ $PGF_2\alpha$ and ovsynch groups. Thereafter the values dropped basal values during induced estrus when artificial insemination was done. The levels significantly increased ($P\leq0.05$) on day 10 of estrus cycle in all the groups. In control group also the mean P_4 concentration was lowest (0.43±0.17 ng/ml) on the day of spontaneous estrus/AI, which rose significantly on day 10. The serum P_4 profiles observed in the study with respect to CIDR and Ovsynch protocols corroborated with the earlier observations of Patel *et al*. (2013) and Manokaran *et al*. (2020). Progesterone concentrations following ovulation have been positively correlated to the volume of uterine secretions, conceptus development, the embryos ability to secrete IFN-τ, embryo viability for subsequent survival and perhaps most importantly conception rates (Manokaran *et al*., 2020). The progesterone concentration obtained in the experimental animals in the study also proved this.

Table 2: Mean (±SE) serum progesterone concentration (ng/ml) on different days of estrus synchronization protocols in Kangayam cows.

	On the day of selection	On the day of $PGF_2\alpha$ injection	On the day of AI	10 days post AI
Group I (Single $PGF_2\alpha$)	0.79^{p}±0.11	2.39^{qa}±0.32	0.73^{p}±0.12	2.88^{qa}±0.12
Group II (Double $PGF_2\alpha$)	0.74^{p}±0.07	3.35^{qb}±0.16	0.70^{p}±0.04	3.34^{qb}±0.14
Group III (CIDR+ $PGF_2\alpha$)	0.53^{p}±0.05	3.14^{qb}±0.16	0.65^{p}±0.06	3.21^{qb}±0.19
Group IV (Ovsynch)	0.62^{p}±0.05	3.31^{qb}±0.16	0.85^{p}±0.06	3.37^{qb}±0.19
Group V (Control)	0.69^{p}±0.07	-	0.81^{p}±0.05	3.18^{qb}±0.13

Means bearing different superscripts (p, q) on different days of blood collection in the same row differ significantly ($P\leq0.05$).

Means bearing different superscripts (a, b) between rows within a column differ significantly ($P\leq0.05$).

Table 3: Mean (±SE) serum estradiol-17β concentration (pg/ml) on different days of estrus synchronization protocols in Kangayam cows.

	On the day of selection	On the day of $PGF_2\alpha$ injection	On the day of AI
Group I (Single $PGF_2\alpha$)	22.04^{y}±0.43	12.92^{x}±0.35	24.21^{zb}±1.74
Group II (Double $PGF_2\alpha$)	23.15^{y}±0.30	13.68^{x}±0.30	25.57^{zc}±0.80
Group III (CIDR+ $PGF_2\alpha$)	22.09^{y}±0.41	13.32^{x}±0.34	24.96^{yb}±1.58
Group IV (Ovsynch)	21.09^{y}±0.41	12.95^{x}±0.34	24.48^{zb}±1.58
Group V (Control)	23.64±0.15	-	22.66^{a}±1.13

Means bearing different superscripts (x, y, z) on different days of blood collection in the same row differ significantly ($P\leq0.05$).

Means bearing different superscripts (a, b, c) between rows within a column differ significantly ($P\leq0.05$).

The serum estradiol-17β concentration was analyzed on the day of selection, on the day of $PGF_2\alpha$ injection (estrus induction) and on the day of artificial insemination and is depicted in the Table 3. The serum estradiol-17β concentration was higher during selection (21.09±0.41 to 23.15±0.30 pg/ml) and during insemination (24.21±1.74 to 25.57±0.80 pg/ml). At the same time, estradiol-17β concentration has fallen down during $PGF_2\alpha$ injection (12.81±0.25 to 13.68±0.30 pg/ml) indicating luteal phase during which the value of serum P_4 ranged from 2.88±0.12 to 3.37±0.19 ng/ml. When compared to this study, higher serum estradiol-17β concentration was recorded by Manokaran *et al*. (2018) in repeat breeder cows treated with ovsynch and ovsynch+vitamin A protocol. The results of the study indicated that the estradiol-17β concentration was higher during induced estrus than the natural estrus in the experimental animals with significant difference ($P \leq 0.05$) as observed by Selvaraju *et al*. (2008). This might be the reason for the more intense and intermediate estrus intensities observed in treatment group animals than control group animals. In control group animals, there was no much variation was observed in the estradiol-17β concentration between the day of selection (23.64±0.15 pg/ml) and day of insemination (22.66±1.13 pg/ml). This could be the reason for higher percentage of weak estrus intensity observed in control group. The secretary pattern of serum estrogen in this study indicated that the synchronization of estrus protocols effective in controlling follicular development and subsequent ovulation in cows.

CONCLUSION

In conclusion, all the four estrus synchronization protocols (Single $PGF_2\alpha$, Double $PGF_2\alpha$, CIDR+ $PGF_2\alpha$ and Ovsynch) can be used with good conception rates in indigenous Kangayam cows. The best results can be obtained by using Double $PGF_2\alpha$ and Ovsynch protocol followed by CIDR+ $PGF_2\alpha$ protocol under field condition. The significant benefits of genetic improvement and reproductive management can be gained in Kangayam animals by implementation of estrus synchronization at field level to increase the population of this pride indigenous breed of Tamil Nadu.

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Conflict of Interest: None

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