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Evaluation of Ovsynch Estrus Synchronization in Holstein Friesian and Holstein Friesian-Jersey Crossbred Cows at District Thatta, Sindh, Pakistan

Sajid Hussain Shah[1], Akeel Ahmed Memon[1]*, Asmatullah Kaka[1], Ahmed Nawaz Tunio[2], Abdullah Channo[1], Qudratullah Kalwar[4], Muhammad Ibrahim Panhwar[3], Kashif Ali Malak[1] and Baban Ali Rahoo[1]

[1]*Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, 70060*
[2]*Department of Surgery and Obstetrics, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, 70060*
[3]*Department of Animal Breeding and Genetics, Faculty of Animal Production and Technology, Shaheed Benazir Bhutto University of veterinary and Animal Sciences, Sakrand, 67210.*
[4]*Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, 67210*

ABSTRACT

The current study was conducted to assess the ovsynch estrus synchronization in imported pure Holstein Friesian and Holstein Friesian-Jersey crossbred cows at district Thatta Sindh. A total 30 Holstein Friesian and Holstein Friesian-Jersey crossbred cattle were selected and divided into three groups i.e., A (10 pure Holstein Friesian cows), B (10 Holstein Friesian-Jersey crossbred cows), and C (5 pure Holstein Friesian and 5 Holstein Friesian–Jersey crossbred cows) as control group. All selected animals were scanned through ultrasound to ensure the normality of reproductive tract. Animals of group A and group B were treated with Ovsynch protocol {GnRH; (Dalmarelin, FATRO) intramuscular at day 0 followed by PGF2α (Dalmazin; FATRO) intramuscular at day 7 and 2[nd] GnRH intramuscular at day 9} for estrus synchronization while animals of group C was kept as untreated control (intramuscular injection of normal saline as placebo on day 0, 7 and 9). Animals of all group underwent fixed time insemination at 16 h of last treatment. Results showed that estrus response vary significantly among the groups ($P<0.05$). Overall estrus response was observed significantly higher in group B (80%), followed by A (60%) and C (10%). Intensity of estrus was determined by observing the estrus signs. Results showed significantly higher intensity of estrus signs in Group B than A and C for mucus discharge 90%, 65%,10%, flehmen 90%, 60%,10%, standing heat80%, 60%, 10% and sniffing vagina 80%, 70%, 10%, respectively. Estrus duration differs significantly among the treatment groups ($P<0.05$). Significantly maximum estrus duration was noted in group B (15.02 ± 0.21 h), then A (13.58 ± 2.22 h) and C (10 ± 0.00 h). In all groups blood samples were collected from the milk vein on day 0 (the first treatment), day 7, day 11 (one day after artificial insemination), day 18 and day 30 from all groups for determining progesterone level. Statistical analysis showed non-significant differences in progesterone level among groups at day 0. Furthermore, on Day 7, Group C differed significantly in progesterone level than A and B; however non-significant difference were observed in group A and B, while in group C difference was significant. On day 11 significantly highest values of blood progesterone were seen in group C (2.63 ± 0.22) followed by A (1.65 ± 0.35) and then B (1.48 ± 0.22). Statistical analysis showed that group C was significantly different from A and B, but there was non-significant difference between A and B. At day 18 highest P4 values were seen in B (4.20 ± 0.44) followed by A (3.33 ± 0.39) and then C (1.88 ± 0.59). Statistical analysis showed that group B and A had non-significant difference among each other, while both groups had significant differences with group C. Blood progesterone level for all the three groups on day 30 showed highest values in group B (10.90 ± 2.44) followed by A (8.28 ± 3.33) and C (4.53 ± 0.44). Statistical analysis showed significant difference among all three groups on day 30. It was concluded that in term of estrus response, cyclic activities and fertility rate, Ovsynch protocol was more effective in Holstein Friesian- Jersey cross breed cows than in Holstein Friesian pure cows.

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SHS, AAM, AK, ANT and AC conceptualized and designed the manuscript. AC and AK analyzed the data. SHS, AAM, AK and AC wrote the manuscript. QK, MIP, KAM and BAR helped in the guidelines and support.

Key words
Ovsynch, Estrus synchronization, Holstein Friesian, Blood progesterone, Ultrasonography

* Corresponding author: akeelahmedmemon@gmail.com
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INTRODUCTION

Energy demands for more milk production and multiple stressors to which the animals are subjected during intensive production conditions are directly linked to a decline in the reproductive efficiency of the cows in more-producing dairy cows (Dejan *et al.*, 2015). The management, wellbeing, and physiology of cows all play a role in reproductive productivity. In dairy cattle, the relationships between nutrition, hormonal systems, and altered reproduction are becoming increasingly clear (Sartori *et al*., 2010). Various techniques have been used to regularize cyclicity in cattle, with estrus synchronization being one of the most effective. Synchronization of estrus programs based on PGF2α and GnRH have been improved to promote follicular growth, luteal lifespan, and ovulation synchrony around artificial insemination (AI). Ovulation before the first GnRH injection boosts estrus cycle synchronization and shortens the time of follicular dominance, all of which are linked to more pregnancies per artificial insemination (P/AI) (Sartori *et al*., 2010).

The Ovsynch protocol, on the other hand, was developed mainly to improve submission rate rather than pregnancy rate, since the artificial insemination (AI) conception rate after Ovsynch is typically within the range recorded for cows inseminated after an observed heat (Abdel *et al*., 2019). In the modern beef industry, estrus synchronization and artificial insemination can also be used to enhance the reproductive potential of cows for a higher pregnancy rate. Furthermore, synchronization of estrus in cows makes artificial insemination at a set time easier (Kasimanickam *et al*., 2009).

The Ovsynch program, which includes 2 injections of a GnRH precursor separated by a single injection of PGF2α, is mainly for dairy cows (Pursley *et al*., 1995). Because synchronization is likely to be used in castte at any phase of the estrous cycle in the field, combining GnRH and prostaglandin results in higher homogeneity of ovarian follicular status at the time of luteolysis induction. In about 85 percent of cows, the first administration of GnRH occurs at a random phase of the estrous cycle and induces either ovulation or luteinization of a dominant follicle (Pursley *et al*., 1995).

The ovsynch method is a GnRH–PGF2α-GnRH therapy sequence that has become prevalent in cattle for estrus synchronization in the previous decade, resulting in appropriate fertility to timed AI (TAI) (Pursley *et al*., 1997). The GnRH injection, on the other hand, induces a large amount of LH to be released, which ovulates a present dominant follicle or induces luteinization of non-viable follicles, resulting in the production of a new follicular wave two or three days later (Twagiramungu *et al*., 1995). P4 levels will stay high after ovulation of the dominant follicles, so PGF2α is given on day seven to cause break down of CL and encourage ovulation of the follicle of the new wave of follicular development. After 48 h of PGF2α injection, a second GnRH injection is recommended for enhanced ovulation synchronization and artificial insemination AI (De-Rensis and Peters, 1999).

The cow and buffalo produce 14 and 10 litres of milk every day, respectively. It is still 5 to 6 times lower than in the developed countries. It's value noting that eight milking animals in Pakistan produce the same amount of milk as one milking animal in the developed countries (Siddiqui *et al*., 2017).

In order to obtain more milk production, the milk composition in Pakistan changed between 1985 and 1986, with a small increase in cow milk and a decline in buffalo milk (67 percent buffalo, 31 percent cow in 1985-86, to 60 percent buffalo, 37 percent cow in 2019-20) (GOP, 2019-20). Local cattle breed of Pakistan are low milk producer as compared to imported dairy cows. The Red Sindhi is a heat-tolerant milk cattle breed that originated in Pakistan's Sindh area. The breed is darker than Sahiwal and has a characteristic red colour. The cattle's milk yield varies between 1100 and 2600 kg every lactation, with an average of 1840 kg per lactation. The fat content of milk fluctuates between 4 and 5.2 percent, with an average of 4.5 percent. Sahiwal cattle are considered to be one of Pakistan's greatest milk cattle breeds. The Sahiwal district of Punjab in Pakistan is where the breed gets its name. The cows are brownish red in colour, with tints ranging from mahogany to greyish red. Bulls extremities are darker than the rest of their bodies. Lactation yields range from 1600 to 2000 (Siddiqui *et al*., 2017).

The Holstein-Friesian is the largest dairy breed, capable of producing 9000-10,000 Kg of milk per lactation on average (305 days). The breed originated in the Netherlands' northern provinces of North Holland and West Friesland. Jersey cattle are a British breed of tiny dairy cattle native to the British Channel Islands of Jersey (Fontanesi *et al*., 2011). According to the American Jersey Cattle Association (AJCA), compared to the Holstein-Friesian cow, Jersey contains more fat, protein, calcium and phosphorus, along with high amounts of vitamin B12. The lactation yield ranges from 5000 to 7500 Kg.

The conception rate in Holstein Friesian cows treated with Ovsynch protocol, Ov-cosynch, Double synch, Heat synch and control as 52.05%, 47.82%, 41.66%, 36.17% and 34.41% respectively (Madhumeet *et al*., 2019). The conception rate in primiparious Holstein Friesian and Jersey cattle in United States inseminated on natural heat were 34% and 40%, respectively (Norman *et al*., 2009).

Farmers in Pakistan's commercial dairy industry imported exotic breeds like Holstein-Friesian and Jersey cattle, as well as their crosses, to achieve high milk yield and milk fat percentage. At our location, only a small amount of research has been done on the estrus synchronization Ovsynch protocol/ response in imported cattle. As a result, the current research will look into the reproductive performance of imported Holstein Friesian and Jersey cows using the Ovsynch protocol in the subtropical climate of Thatta Sindh Pakistan. In our local circumstances, it is hypothesized that the results of the Ovsynch estrus synchronization protocol in imported Holstein Friesian and Jersey crossbred cows would be distinct. As a result, the following were the goals of my research.

MATERIALS AND METHODS

Experimental design

A total 30 animals of Holstein Friesian-Jersey and Holstein Friesian cattle were divided into three groups each of 10: A (HF, Holstein Friesian cows), B (HF-J, Holstein Friesian-Jersey cows), and C (Holstein Friesian and Holstein Friesian-Jersey crossbred cows). For estrus synchronization, animals in this group A and B were given Ovsynch (GnRH intramuscular on day zero, PGF2α intramuscular on day seven, and 2nd GnRH intramuscular on day nine). The animals of group C were injected with 2ml intramuscular normal saline (FDL Pakistan) on days 0, 7, and 9 of the experiment.

Ovsynch treatment

The Ovsynch protocol used to treat each animal is as follows.

On day 0 (09:00 am) 50 g lecirelin acetate, a long-acting synthetic analogue of GnRH, was administered intramuscularly 2 ml of Dalmarelin, FATRO. On day 7 (09:00 am) 150 g cloprostenol, a synthetic analogue of PGF2α, was administered as 2ml Dalmazin FATRO intramuscularly. On day 9 (05:00 pm) 50 g lecirelin acetate, a long-acting synthetic analogue of GnRH, is injected as 2 ml Dalmarelin, FATRO intramuscularly. On day 10 (09:00 am) the animals were artificially inseminated fixed timed AI at a 16-h interval after receiving the second GnRH injection.

Estrus detection

After the last therapy, the cows in groups A, B, and C were closely monitored for behavioral changes to the heat. In all groups, insemination was carried out at 16 h after the last therapy. Estrus intensity was measured 4 times a day at 6 am, 12 pm, and 6 pm, 12 am, for 30 min each time. With some modifications, estrus behaviour was documented using the Van Eerdenburg *et al*. (1996) scale (3 for discharge of valvular mucous, 3 for flehmen process, 5 for restlessness, 10 for vagina sniffing of another cow, 15 for resting of Chin, 35 for non-standing but mounted, 45 for mounting on other cows, and 100 for standing estrus). When a signs was observed multiple times and the total number of points exceeded 100 over the course of a day, the estrus response was deemed robust. The estrus response is called poor if the sum of the points is less than 100.

Pregnancy determination

The pregnancy was be confirmed by ultrasound (Honda Portable veterinary ultrasound system HS-102V Japan, linear rectal probe 5.0 MHz) 45 days post A.I.

Sample collection

On day 0 (the first treatment), day 7, day 11 (one day after artificial insemination), day 18 and day 30 in groups A, B, and C blood samples were drawn from the milk vein using red cap clot activator tubes which contain CAT serum clot activator covered with micronized silica particles that activate clotting when the tubes are gently turned or centrifuged. After collection, blood samples were centrifuged (20 min at 1,600×*g*; 4°C). Serum was collected and stored at −20°C until assayed for P4 concentrations using progesterone ELISA kits, Immunolab Germany. Serum samples were stored in iceboxes and transferred to the CDC Laboratories and Research Center, Chiragh Diagnostic Complex 8-2 D1 Township, Lahore for analysis of blood progesterone concentrations.

Statistical analysis

The data were analyzed using two-way analysis of variance (ANOVA) using Statistix 8.1 version (2006) with a significance level of LSD < 0.05.

RESULTS

Estrus response and duration

Figure 1A and 1B show the estrus response of imported Holstein Friesian and Holstein Friesian-Jersey crossbred cows following ovsynch estrus synchronization. The estrus response and duration differ significantly among the groups ($P<0.05$). Overall estrus response was observed significantly higher in group B (80%), followed by A (60%) and C (10%). Significantly maximum estrus duration was noted in group B (15.02±0.21 h), then A (13.58±2.22 h) and C (10±0.00 h). Table I shows estrus signs in the Holstein Friesian and Holstein Friesian-Jersey crossbred cows after ovsynch estrus synchronization. Results showed significantly higher intensity of estrus signs in group B than A and C for mucus discharge 90%, 65%,10%, flehmen 90%, 60%,10%, standing heat 80%, 60%,10% and sniffing vagina 80%, 70%, 10%, respectively.

Figure 2 shows the blood progesterone level for all three groups at day 0 and according to results highest value was seen in A (1.83±0.31) followed by B (1.75±0.23) and then C (1.42±0.13). Statistical analysis showed non-significant different among groups at day 0. Furthermore, at day 7 highest values were seen in A (5.02±0.63) followed by B (4.68±0.45) and then C (3.13±0.40). Statistical analysis showed non-significant difference between A and B at day 7 while C was found different significantly from A and B. At day 11significantly highest values of blood progesterone were seen in group C (2.63±0.22) followed by A (1.65±0.35) and then B (1.48±0.22). Statistical analysis resulted that group C differ significantly from A and B. However, difference in group A and B was non-significant. At day 18 highest Progesterone values in group B was 4.20±0.44 followed by A 3.33±0.39 and C 1.88±0.59. Statistical analysis showed non-significant difference amongst group B and A. however significant difference were noted among group A, B with group C. Blood progesterone level for all three groups at day 30 showed highest values in group B (10.90±2.44) followed by A (8.28±3.33) and C (4.53±0.44). Statistical analysis showed significant difference among all three groups at day 30.

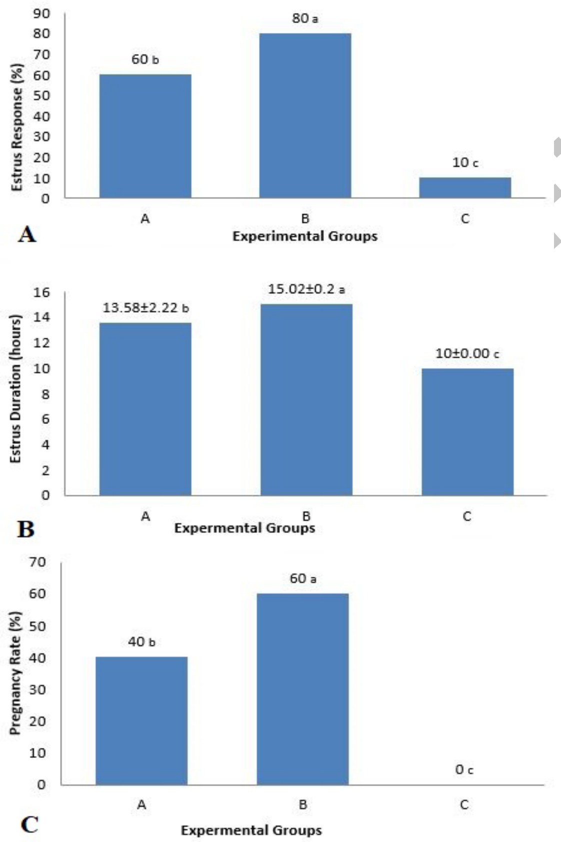

Fig. 1. Effect of ovsynch estrus synchronization protocol on estrus response (A), duration (B) and pregnancy rate (C) in Holstein Friesian and Holstein Friesian-Jersey crossbred cows.

Table I. Effect of ovsynch estrus synchronization on estrus signs of Holstein Friesian and Holstein Friesian-Jersey crossbred cows (%).

Groups	A	B	C (Control)
Total no. of animals	10	10	10
Mucous vulvular discharge	65%	90%	10%
Flehmen	60%	90%	10%
Restlessness	60%	60%	10%
Vagina Sniffing of another cow	70%	80%	10%
Resting of chin	40%	80%	10%
Not standing but mounted	50%	60%	10%
Mounting other cattle	60%	70%	10%
Standing estrus	60%	80%	10%

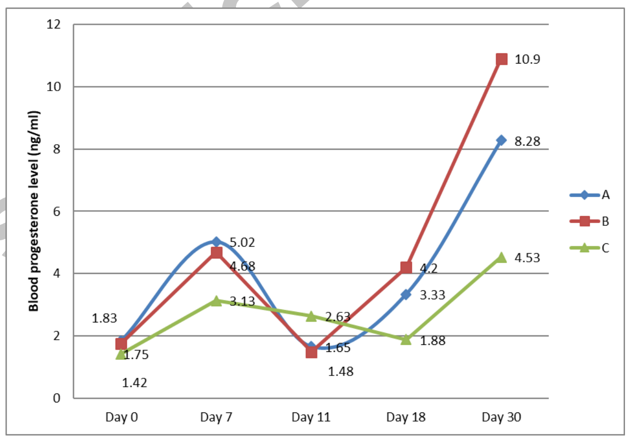

Fig. 2. Effect of ovsynch estrus synchronization on blood progesterone level in Holstein Friesian and Holstein Friesian-Jersey crossbred cows.

Pregnancies were determined through ultrasound at day 45 (Fig. 1C). Pregnancy percentages were recorded as 40% and 60% in group A and B, respectively. However, not a single animal was found pregnant in control group C. Statistical analysis showed significant difference in pregnancy rate among the treatment groups ($P < 0.05$).

DISCUSSION

In present study response of Ovsynch protocol of estrus synchronization were investigated in. The findings revealed that Ovsynch protocol was much effective in Holstein Friesian- Jersey cross cows as compared to Holstein Friesian pure breed cows in terms of estrus response, estrus duration and pregnancy rate.

Estrus synchronisation and fixed-time artificial insemination (AI) is a beneficial technique in breeding management, particularly in dairy cattle, because it enhances heat detection efficiency and dairy farm profitability (Jayaganthan *et al*., 2016). Synchronization of estrus has been created to assist producers in optimal reproductive management (Pursley *et al*., 1995). Because of the poor estrus detection frequently seen in high-producing cows, the Ovsynch protocol is routinely used in dairy farms (Santos *et al*., 2004). Pursley *et al*., (1995) modified the Ovsynch protocol (day 0 GnRH, day 7 prostaglandin PGF2, day 9 GnRH, day 10 timed AI). Programmers based on GnRH and PGF2 have been refined to optimize follicular growth, luteal duration, and ovulation synchronization around the time of AI. The ovsynch regimen is a series of GnRH-PGF2-GnRH therapies that have been popular in cattle over the last decade for estrus synchronization, resulting in acceptable fertility to timed AI (TAI) (Jabeen *et al*., 2012). The Ovsynch regimen's reproductive response, on the other hand, is determined by the moment in the cycle when the first GnRH is administered. Starting the programmed during mid diestrus (days 5–12 of the cycle) improves conception rates in nursing dairy cows by increasing first wave ovulation (initiation on days 5–9) and the number of cows with high progesterone (>1 ng/ml) at the time of the PGF2 injection (Santos *et al*., 2004).

In the current study estrus response was significantly higher with ovsynch estrus synchronization protocol in Holstein Friesian-Jersey cross cows group B (80%) than Holstein Friesian pure breed cow group A (60%) and control group C (10%). Small *et al*. (2001) reported that 51.5% estrus response in Holstein cow through ovsynch estrus synchronization protocol. Similarly, Dhami *et al*. (2015) reported that estrus response in crossbred cows was 100% in CIDR, Ovsynch, and Norgestomet ear implant protocols. Increased blood concentrations of somatotropin and prolactin, lactation stimulators, and decreased insulin, a hormone that is antagonistic to lactation and may be important for normal follicular development, have been linked to lower reproductive performance in high-producing Holstein Friesian cows compared to crossbred cows (Nebel *et al*., 1994). Increased blood concentrations of somatotropin and prolactin, lactation stimulators, and decreased insulin, a hormone that is antagonistic to lactation and may be important for normal follicular development, have been linked to lower reproductive performance in high-producing Holstein Friesian cows compared to crossbred cows (Nebel *et al*., 1994).

The duration of estrus was non significantly higher for control group B (15.02± 0.21 h) than A (13.58 ± 2.22 h) and C (10 ± 0.00 h). There was only 1 animal from control group C which was found in estrus after 12 h of end of protocol at day 10 and was inseminated at standing heat. The estrus duration of that animal was 10 h. Results of this study are in accord with Britt *et al*. (1986) who reported estrus duration in 13 Holstein (2 primiparious and 11 multiparious) as 13.8 h on dirt and estrus duration reported in dairy cattle as 11± 0.7 h (Richardson *et al*., 2002). Estrus duration in present study are similar as reported by Roelofs *et al*. (2005) who investigated 13.6 h in primiparious cows. This difference in heat duration may be due to heat observation method, housing types, breed and housing management etc.

Estrus indications were seen in all groups, however group B had the most (80%) compared to group A (60%) and group C (0%). (10%). Mucus discharge (65 percent), (90%), (10%), flehmen (60%), (90%), (10%), standing heat (60%), (80%), (10%), and sniffing vagina of other animals (70%, 80%), (10%) were the most frequently observed estrus signs in groups A, B, and C, respectively. Pure Holstein Friesian cows provide more milk than Holstein Friesian-Jersey hybrid cows. High energy demands for high milk production, as well as many stressors to which the animals are exposed during intensive production conditions, are directly associated to a decrease in the reproductive efficiency of dairy cows (Dejan *et al*., 2015). As a result, the Ovsynch estrus synchronization protocol proved extremely successful.

The overall pregnancy rate was (40%), 4 animals in group A and (60%), 6 animals in group B were found pregnant while not a single animal was found pregnant in control group C. Similar results were reported that pregnancy rate with ovsynch was 39.7% at day 30 in pure Holstein cow and 54.54% in crossbred Jersey cow (Shahzad *et al*., 2020). Pregnancy rate with ovsynch was investigated in pure Holstein cow as 55% (Karki *et al*., 2018) and 52.5% (Madhumeet *et al*., 2019). Cirit *et al*. (2008) investigated 50% pregnancy rate in primiparious Holstein Friesian pure cow while similar results were reported as 53.6% pregnancy rate in primiparious Holstein Friesian pure cow and 50% pregnancy rate in multiparious Holstein Friesian pure cow on pregnancy diagnosis at day 31 after insemination (Keskin *et al*., 2011). Seasonal factor also effect on pregnancy rate as 57.7% in winter season and 38.1% in summer in Holstein Friesian cow due to heat stress which reduces reproductive performance of Holstein Friesian cow (Keskin *et al*., 2011). The present study was conducted in second week of February 2021 to achieve good results through estrus synchronization.

The blood P4 level higher from day zero to 7 in group A, B and C, respectively. For all three groups at day 0 according to results highest value was seen in A (1.83± 0.31) followed by B (1.75± 0.23) and then C (1.42± 0.13). At day 0 all the values were non significantly different from each other because all selected animals in each group were in early diestrus phase. For all three groups at day 7 highest values were seen in A followed by B and then C. Statistical analysis revealed that A and C were found different significantly but B was non significantly different from A and C. Progesterone level declined at day 11 in all groups as (1.65± 0.35), (1.48± 0.22), (2.63± 0.22) in group A, B and C, respectively because of luteinization of corpus luteum by given PGF2 at day 7 in group A and B while in control group C only 1 animal shows estrus at day 10 so mean progesterone level of group C also declined. At day 10 animals of group A (60%), B (80%) and C (10%) showed estrus signs. Blood progesterone level for all three groups at day 11 was shown in table and according to results highest values were seen in C followed by A and then B. Statistical analysis resulted that C was found different significantly from A and B but among A and B non-significant difference was found. Then again it increased from day 18 to day 30 as (3.33± 0.39), (4.20± 0.44) to (8.28± 3.33), (10.90± 2.44) in group A and B, respectively while in group C it decreased at day 18 as (1.88± 0.59) and increased at day 30 as (4.53± 0.44). At day 18 highest values were seen in B followed by A and then C. At day 18 B and C were found significantly different from each other but A had non-significant difference with B and C. At day 30, highest values were seen in B followed by A and then C. According to statistical analysis all the values had significant difference among them. In control group C at day 18 progesterone level decreased because animals selected for experiment were in early luteal phase so that cyclic animals were in follicular phase of estrus cycle at day 18. Our results are similar as reported by Dhami *et al*. (2015) who investigated blood progesterone level in crossbred cows at day 0 as 1.70± 0.34 ng/ml and day 7 as 4.10± 0.78 ng/ml. Similarly, Vukovic *et al*. (2015) reported that blood progesterone level of pure Holstein cow in ovsynch treatment was 13.6± 2.8 ng/ml in pregnant and 4.5± 0.7 ng/ml in non-pregnant animals on 21 days post AI which were similar to our current results. Some animals showed blood progesterone level > 10 ng/ml at day 30 and were considered pregnant while < 5.4 ng /ml at day 30 considered as non pregnant. At day 30 the mean progesterone level in group C was 4.53 because all animals of group A, B and C selected for experiment were in early luteal phase at start of experiment and at day 30 most of animals of group C were again in luteal phase.

In present study, rate of pregnancy was higher as 60% in crossbred animals (Holstein Friesian-Jersey cross) as compared with purebred animals (Holstein Friesian) as 40% and these results were in similar with Bage (2002), who concluded that overall pregnancy rate of 55% was greater in crossbred animals treated with Ovsynch than of purebred Holstein 35.1% treated with Ovsynch. Producers can employ a variety of reproductive technologies, including as ovulation synchronization and timed insemination procedures, to automate reproductive management. Users of these technologies must know which programs are the most practical, handy, and effective. The Ovsynch/timed artificial insemination is estrus synchronization procedure in reproductive biology that has been advised to help nursing dairy cows reproduce better in short time (Rabiee *et al*., 2005). One of the most important aspects of a profitable dairy system is reproductive efficiency. Milk cattle's reproductive performance is harmed by physiological and environmental stressors such as excessive milk output, insufficient nutritional intake, low body condition, and intense management methods (Thatcher *et al*., 2000).

CONCLUSIONS

It was concluded that the estrus response was higher in Ovsynch estrus synchronization protocol in Holstein Friesian-Jersey cross breed animals as compared to Holstein Friesian pure animals in term of estrus signs. However, Estrus duration was also longer in Holstein Friesian- Jersey cross breed animals as compared to Holstein Friesian pure animals when treated with Ovsynch estrus synchronization protocol. Meanwhile, Ovsynch estrus synchronization also improved pregnancy rate in Holstein Friesian- Jersey cross breed animals as compared to Holstein Friesian pure animals.

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IRB approval

The research work was approved by the Departmental Board of Studies (BOS) of Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University on 18th November 2021.

Ethical statement

The research was done with integrity, fidelity, and honesty by following all rules and regulations.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abdel, T.R., Khalil, Y., and Hussein, M.M., 2019. Pregnancy outcome of lactating dairy cows assigned for Presynch-Ovsynch synchronization program and inseminated either at detected standing heat or at scheduled fixed time. *Asian Pac. J. Reprod.*, **8**: 181-186. https://doi.org/10.4103/2305-0500.262835

Bage, R., 2002. *On repeat breeding in dairy heifers: With special focus on follicular dynamics, ovulations, and oocyte quality.* Doctoral thesis, Swedish University of Agricultural Sciences, Sweden. pp.139.

Britt, J.H., Scott, R.G., Armstrong, J.D., and Whitacre, M.D., 1986. Determinants of estrous behavior in lactating Holstein cows. *J. Dairy Sci.,* **69**: 2195-2202. https://doi.org/10.3168/jds.S0022-0302(86)80653-1

Cirit, A.U., Bacinoglu, A.S., Taş, B.M., Demir, A.K., Baş, C.A., and Akari, I.K., 2008. Evaluation of short estrus synchronization methods in dairy cows. *Anim. Reprod. Sci.*, **109**: 65–76. https://doi.org/10.1016/j.anireprosci.2007.12.004

Dejan, S., Tadej, V., Ocepek, M., and Marjan, J., 2015. Fertility in high-producing dairy cows: Reasons for decline and corrective strategies for sustainable improvement. *Soc. Reprod. Fertil. Suppl.,* **64**: 237-254.

De-Rensis, F., and Peters, A.R., 1999. The control of follicular dynamics by PGF2α, GnRH, hCG and oestrus synchronization in cattle. *Reprod. Domest. Anim.*, **34**: 49-59. https://doi.org/10.1111/j.1439-0531.1999.tb01383.x

Dhami, A.J., Nakrani, B.B., Hadiya, K.K., Patel, J.A., and Shah, R.G., 2015. Comparative efficacy of different estrus synchronization protocols on estrus induction response, fertility and plasma progesterone and biochemical profile in crossbred anestrus cows. *J. Anim. Sci.*, **8**: 1310-1316. https://doi.org/10.14202/vetworld.2015.1310-1316

Fontanesi, L., Scotti, E., and Russo, V., 2011. Haplotype variability in the bovine MITF gene and association with piebaldism in Holstein and Simmental cattle breeds. *Anim. Genet.*, **43**: 250-256. https://doi.org/10.1111/j.1365-2052.2011.02242.x

GOP, 2019-20. *Pakistan economic survey. 2019-2020*. Government of Pakistan, Finance Division, Economic Advisors Wing, Islamabad, pp. 35-36.

Jabeen, S., Anwar, M., Rabi, S.H., Mehmood, A., Murtaza, S., and Shahab, M., 2012. Determination of ovsynch efficiency for estrus synchronization by plasma LH and P4 Levels in Niliravi buffalo during Peak and low breeding season. *Pak. Vet. J.,* **33**: 221-224.

Jayaganthan, P., Vijayarajan, A., Prabaharan, V.P., and Sivakumar, A., 2016. Synchronization of ovulation in repeat breeding crossbred jersey cows using GnRH and PGF2 alpha. *Int. J. Cogn. Technol.*, **5**: 2377-2381.

Karki, B., Raut, R., Sankhi, K.P., Mandal, U., and Gautam, G., 2018. Fertility improvement by ovsynch protocol in repeat breeder cattle of Kathmandu valley. *Int. J. appl. Sci. Biotechnol.*, **6**: 261-264. https://doi.org/10.3126/ijasbt.v6i3.21183

Kasimanickam, R., Hall, J.B., Currin, J.F., and Whittier, W.D., 2009. Sire effect on the pregnancy outcome in beef cows synchronized with progesterone based Ovsynch and CO-Synch protocols. *Anim. Reprod. Sci.*, **104**: 1-8. https://doi.org/10.1016/j.anireprosci.2007.01.003

Keskin, A., Mecitoglu, G.Y., and Gumen, A., 2011. Comparison of responses to ovsynch between Holstein-Friesian and Swedish red cows. *J. Dairy Sci.*, **94**: 1784-1789. https://doi.org/10.3168/jds.2010-3579

Madhumeet, S., Sharma, A., Kapse, S., Kashyab, A., and Kumar, P., 2019. Efficacy of different estrus synchronization protocols in repeat breeder cows. *Indian J. Anim. Sci.*, **89**: 958-960.

Nebel, R.L., Walker, W.L., McGilliard, M.L., Allen, C.H., and Heckman, G.S., 1994. Timing of artificial insemination of dairy cows: Fixed-time once daily versus morning and afternoon. *J. Dairy Sci.*, **77**: 3185–3191. https://doi.org/10.3168/jds.S0022-0302(94)77261-1

Norman, H.D., Wright, J.R., Kuhn, M.T., Hubbard, S.M., Cole, J.B., and VanRaden, P.M., 2009. Genetic and environmental factors that affect gestation length in dairy cattle. *J. Dairy Sci.,* **92**: 2259-2269. https://doi.org/10.3168/jds.2007-0982

Pursley, J., Kosorok, M.R., and Wiltbank, M.C., 1997. Reproductive management of lactating dairy cows using synchronization of ovulation. *J. Dairy Sci.*, **80**: 301-306. https://doi.org/10.3168/jds.S0022-0302(97)75938-1

Pursley, J., Mee, M., and Wiltbank, M.C., 1995. Synchronization of ovulation in dairy cows using PGF2α and GnRH. *Theriogenology*, **44**: 915-923. https://doi.org/10.1016/0093-691X(95)00279-H

Rabiee, A.R., Lean, I.J., and Stevenson, M.A., 2005. Efficacy of ovsynch program on reproductive performance in dairy cattle: A meta-analysis. *J. Dairy Sci.,* **88**: 2754-2770. https://doi.org/10.3168/jds.S0022-0302(05)72955-6

Richardson, A.M., Hensley, B.A., Marple, T.J., Johnson, S.K., and Stevenson, J.S., 2002. Characteristics of estrus before and after first insemination and fertility of heifers after synchronized estrus using GnRH, PGF2α, and progesterone. *J. Anim. Sci.*, **80**: 2792-2800. https://doi.org/10.2527/2002.80112792x

Roelofs, J.B., Van Eerdenburg, F.J.C.M., Soede, N.M., and Kemp, B., 2005. Various behavioral signs of and their relationship with time of ovulation in dairy cattle. *Theriogenology*, **63**: 1366-1377. https://doi.org/10.1016/j.theriogenology.2004.07.009

Santos, J.E.P., Thatcher, W.W., Chebel, R.C., Cerri, R.L.A., and Galvao, K.N., 2004. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. *Anim. Reprod. Sci.*, **82**: 513–535. https://doi.org/10.1016/j.anireprosci.2004.04.015

Sartori, R., Bastos, M.R., and Wiltbank, M.C., 2010. Factors affecting fertilization and early embryo quality in single and super ovulated dairy cattle. *Reprod. Fertil. Dev.*, **22**: 151-158. https://doi.org/10.1071/RD09221

Shazad, A.H., Sattar, A., Ahmad, N., Ahmad, I., Yousaf, M.S., Abbas, S., and Nak, D., 2020. Evaluation of synchronization protocols and methods of early pregnancy diagnosis in dairy cattle. *South Afr. J. Anim. Sci.*, **6**: 50. https://doi.org/10.4314/sajas.v50i6.4

Siddiqui, A.R., Ginther, O.J., Araujo, E.R., and Dangudubiyyam, S.V., 2017. Follicles and gonadotropins during waves 2 and 3 in three-wave interovulatory intervals in Bos taurus heifers. *Theriogenology*, **104**: 192-197. https://doi.org/10.1016/j.theriogenology.2017.08.019

Small, J.A., Ambrose, D.J., McCaughey, W.P., Ward, D.R., Sutherland, W.D., Glover, N.D., and Rajamahendran, R., 2001. The effects of gonadotrophin releasing hormone in prostaglandin F2a-based timed insemination programs for beef cattle. *Can. J. Anim. Sci.*, **81**: 335–343. https://doi.org/10.4141/A00-077

Thatcher, W.W., Mattos, R., Moreira, F., Binelli, M., and Ambrose, J.D., 2000. Experimental manipulation of follicular growth. *Reprod. Domest. Anim.,* **6**: 27-33.

Twagiramungu, H., Guilbault, L.A., and Dufour, J.J., 1995. Synchronization of ovarian follicular waves with a gonadotropin-releasing hormone agonist to increase the precision of estrus in cattle. *J. Anim. Sci.*, **73**: 3141-3151. https://doi.org/10.2527/1995.73103141x

Van-Eerdenburg, F.J.C.M., Loeffler, H.S.H., and Van-Vliet, J.H., 1996. Detection of estrus in dairy cow: A new approach to an old problem. *Veterinary Q.*, **18**: 52-54. https://doi.org/10.1080/01652176.1996.9694615

Vukovic, D., Bozic, A., Relic, R., Stancic, B., Gvozolic D., and Kucevic, D., 2015. Progesterone concentration in milk and blood serum and reproductive efficiency of cows after Ovsynch treatment. *Turk. J. Vet. Anim. Sci.*, **40**: 75-80. https://doi.org/10.3906/vet-1505-17