Journal of Animal & Veterinary Advances
MEDWELL PUBLICATIONS

Development of Fixed-Time Artificial Insemination Through Luteinizing Hormone Peak, Plasma Progesterone and Follicular Dynamics in Locally Adopted *Bos indicus* Cow of Azad Jammu and Kashmir

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Key words: Estrus synchronization, Ovsynch, CIDR, LH surge, progesterone, non-descript cows

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Page No.: 82-91
Volume: 19, Issue 6, 2020
ISSN: 1680-5593
Journal of Animal and Veterinary Advances

Abstract: The experiments were conducted aiming to evaluate the effect of three estrus synchronization protocols viz. Ovsynch, CIDR and CO-Synch+CIDR on LH Peak, Plasma P4 and follicular dynamics in a fixed-time AI (FTAI) in locally adopted non-descript indigenous cows. The blood samples were collected at 2 h intervals, starting at 12-108 h from post $PGF_{2\alpha}$ to determine the LH surge and for P4 on day 0, 4, 7, 11 and then once weekly for 8 weeks of estrus synchronization and FTAI. A better synchrononyof LH peak was observed in Ovsynch group (48.40±0.40 h post $PGF_{2\alpha}$) compared to CIDR (71.67±9.74 h) and CO-Synch+CIDR (54.86±4.71 h) protocols. The mean follicular diameter, daily growth rate and ovulation rate did not differ ($p>0.05$) among three groups. The interval between $PGF_{2\alpha}$ and ovulation was lower ($p>0.05$) in Ovsynch group when compared to CIDR and CO-Synch+CIDR groups. The conception rate at first service (80%) in Ovsynch group was significantly higher ($p<0.05$) compared to CIDR and CO-Synch+CIDR groups. It is concluded that the time of LH peak in synchronized estrus of non-descript indigenous cows is simsilar to taht in *Bos Taurus*cows and Ovsynch protocol induced the best synchrony of pre-ovulatory LH surge and ovulation.

INTRODUCTION

The indigenous cattle in Pakistan administrative Kashmir are of Bos indicus, require small amount of feed to meet nutrient requirement for maintenance because of small in size. The poor productive performance of indigenous cattle may be because of poor genetic makeup[1]. The application of artificial insemination has

the potential to make genetic improvement in *Bos indicus* cattle by using the semen from pedigree proven bulls. However, the scattered and small scale farms and difficult movement due to hilly terrain are some of the most important obstacles in large scale use of the AI technique in the state. Another problem of dairy industry is lack of accurate estrus detection[2]. Therefor, the artificial insemination relying on natural estrus is a slow process for a rapid genetic improvement. The genetic improvement program can be accelerated by the application of estrus synchronization and Fixed-time Artificial Insemination (FTAI). The additional benefits are the choice of the calving season, shortening the postpartum interval increasing of calf uniformity and accuracy of Artificial Insemination (AI)[3]. It is a reproductive management tool that can increase production efficiency and economic returns in the relative shorter time[4].

Ovsynch protocol has been established that precisely synchronize the time of ovulation by utilizing GnRH and PGF2α[5]. Although, in farm animals the regulation of ovulation by changing or supplementing endogenous progesterone release has been studied extensively for >50 years[6], however, the fertility of induced ovulation is still variable.The CIDR device with GnRH and $PGF_{2\alpha}$ may provide a more efficient protocol of improving fertility (CO-Synch+CIDR) in non-cycling postpartum cows[7]. However, the benefits from this technology can only be obtained if cows have a good body condition and without any reproductive abnormalities[8]. In cows, the measurement of LH hormone in peripheral circulation is helpful to understand the timing of ovulation which could be helpful in improving its fertility[9]. The detailed knowledge of ovarian follicular dynamics[10] permitted the use of hormones to regulate the follicular growth and the beginning of ovulation, thus, allowed FTAI in European (*Bos taurus*)[5] and Zebu breeds (*Bos indicus*)[11]. A synchronization protocol should initiate follicular development by activating hypothalamus-pituitary gonadal axis, overcomes the effect of season on breeding. The Ovsynch protocol became popular for estrus synchronization in cattle, resulting in an acceptable fertility to timed AI[12]. Numerous variations of the protocol have also been tested and developed to meet demands of different physiological situations[13,14].

In cattle the duration of estrus lasts from 6-30 h with an average of 20 h[15]. In our earlier study we have investigated that the average duration of total estrus length in non-descript *Bos indicus* is shorter that of *Bos taurus*[16]. Although, the estrus synchronization and FTAI have been extensively studied in *Bos taurus* cattle in many parts of the world. However, very limited information is available for *Bos indicus* cattle of Pakistan and no work are available on the estrus induction treatments and FTAIin Non-descript cattle particularly in Azad Jammu and Kashmir. Therefore, the hypothesis for the present study was that in non-descript cattle; the time of LH peak in synchronized estrus may differ from that in *Bostaurus* becausee of difeferent estrus behavior. Therefore, the objective was to measure the LH Peak, Plasma P4 and ovulation time in non-descript indigenous cattle (*Bosindicus*) to estabilish timing of fixed-time artificial insemination in synchronized estrus.

MATERIALS AND METHODS

To conduct this study, we enrolled cattle from the Livestock Development Research Centre (LDRC), Muzaffarabad (34.361ºN and 73.662ºE), AJ&K during low breeding season (January-February). The cows were randomly selected regardless the stage of estrous cycle with Body Condition Score (BCS) ranges from 3.0-3.5. BCS with 1 for very thin, 2 for thin, 3 for moderate, 4 for optimal and 5 for very fat was used[17]. Cows without any reproductive problems were selected after screening the reproductive tract using ultrasound macine. All the cows were stall fed with Total Mixed Ration (Big Feed Pvt. Ltd Pakistan) under the similar management and environmental conditions.

Protocols: Eighteen healthy Non-descript cows (n =18) were synchronized with three treatments using viz. Ovsynch (OVS) CIDR alone and iii) CO-Synch+CIDR. In Ovsynch group (n = 5) an injection (2 mL) of 100 mg of GnRH analogue (Dalmarelin; lecirelin acetate 25 mcg mL^{-1}, FATRO S.p.A.-pharmaceutical veterinary Industry, Italy) was given on day 0, Seven days after the GnRH analogue injection, 25 mg of $PGF_{2\alpha}$ (Lutalyse™, Dinoprost tromethamine 5 mg mL^{-1}, Pfizer manufacturing Belgium NV- Puurs-Belgium) injection (i.m) was given to induce luteolysis. The second dose of GnRH was administered on day 9 and FTAI was performed at 16 h after second GnRH injection. In CIDR alone group, the progesterone impregnated device (1.38 g of progesterone, Pfizer New Zealand Ltd.) was inserted in the vagina of all the cows (n = 6) on day 0 on day 7 $PGF_{2\alpha}$ was administered and CIDR was removed. TAI was performed at 48 h after CIDR removal. In CO-Synch+CIDR group (n = 7), CIDR was inserted intravaginally and GnRH analogue was administered on day 0. At d 7, CIDR was removed and cow received $PGF_{2\alpha}$. On d 9, second GnRH injection was administered and FTAI was performed 48 h later after CIDR removal. The cows that showed heat 18-21 days post-AI were re-inseminated. About 45 days post-inseminationtrans-rectal ultrasonography was performed by a veterinarian to determine pregnancy status. Rectal palpation was done at 60 day's post-insemination for pregnancy confirmation.

Blood collection and hormonal assays: A blood sample (7 mL) was obtained from the jugular vein of all eighteen

cows. The blood was collected in a heparinized tube at 2-h interval, starting 12 h from $PGF_{2\alpha}$ injection up to 108 h for analysis of LH. T he blood sample for Progesterone (P4) were collected from each cow in all groups on day 0, 4, 7, 11 and then once weekly for 8 weeks of estrus synchronization and TAI program. Immediately. the blood was centrifuged at 1,000×g for 15 min and plasma was stored at -40°C until analyzed. The plasm LH hormone was analysed using a double antibody competitive binding radioimmunoassay with slight modifications in the method as described by Kanai and Ischikawa[18]. The hormone was supplied in lyophilized form by the National Hormone and Peptide Program (NHPP) Harbor-UCLA Medical Center, California, USA. The sensitivity of the assay was 0.78 ng mL^{-1}. The intra-assay and inter-assay coefficients of variation were 3.7 and 4.8%, respectively. Progesterone levels were quantified by solid-phase radioimmunoassay (IM 1188 Beckman Coulter, IMMUNOTECH- Czech Republic). All samples were assayed in duplicate and the sensitivity of the assay was 0.03-50 ng mL^{-1} with intra-and-inter assay coefficients of variation were 8.15 and 8.66%, respectively.

Follicular dynamics: The ovaries of fifteen animals were scanned by trans-rectal ultrasonography using an Aloka SSD-500 ultrasound machine with a 7.5 MHz linear array trans-rectal probe and a real-time B-mode scanner. The ultrasonography was performed at the time of $PGF_{2\alpha}$ injection and then at the interval of 12 h until after the disappearance of the Dominant Follicle (DF) after second GnRH analogue injection. Follicles more than ≥5 mm in diameter were measured. Follicle diameter was determined by averaging diameter at the widest point and at a right angle to the first measurement using the internal calipers on the Aloka SSD-500. The measurement and time of disappearance of the DF and growth rate of the follicle per day were recorded. Maximum diameter of the DF was defined as the diameter just before ovulation while ovulation was defined as the disappearance of a previously recorded large follicle from an ovary.

All the protocols and procedures used are in accordance with the guiding principles for the ethical manner in the care and use of nonhuman animals in research. Approval was taken form the ethical committee of the department for this study.

Statistical analyses: Ovulation time and follicular dynamics reported as mean and Standard Errors of the Mean (SEM) were analyzed by one-way ANOVA. Chi-square tests of independence were used to compare the conception and ovulation rates among different groups. A probability level of $p<0.05$ was considered significant. All the data were analyzed using Graph Pad Prism 6.0 software (Graph Pad Software, Inc., San Diego, CA, USA).

To determine if an LH surge occurred, a baseline mean and Standard Deviation (SD) were calculated for all samples after deleting the 2 largest concentrations. An "LH surge" was defined to occur when the largest LH concentration exceeded the mean of the remaining baseline by 2 SD[19]. Progesterone level of >1.0 ng mL^{-1} on day 18 post GnRH-1 injection was used as evidence of luteal activity[20]. Presumptive conception rate for animals was based on P4 levels <1.0 ng mL^{-1} on day 11 proceeding with an increase to >1 ng mL^{-1} on days 18, 25, 32, 39, 46, 53, 60 and 67.

RESULTS AND DISCUSSION

All cows in Ovsynch group had normal LH profile with a synchronized peak of the LH surge occurring at 48 h in 80.0% and at 50 h in 20.0% cows after the administration of $PGF_{2\alpha}$. The LH peak was observed at 1-3 h after exogenous GnRH. These cows had P4 level >1 ng mL^{-1} on day 18 post-treatment, thus it was assumed that all cows (100%) responded positively to the Ovsynch protocol in term of estrus induction and synchronization of ovulation (Table 1; Fig. 1 and 2). In CIDR group, although all cows showed the LH peak (100%), however, it was not synchronous. Five (83.33%) animals in CIDR alone group were responded positively (Table 2, Fig. 3 and 4). One animal responded with an LH peak at 96 h post-$PGF_{2\alpha}$ however no P4 rise was noted in CIDR alone treatment until 67 days post-$PGF_{2\alpha}$ indicating no ovulation (Fig. 5 and 6). In CO-Synch+CIDR group, three animals out of 7 (42.85%) showed an LH peak at 48 h after $PGF_{2\alpha}$ administration, two (28.57%) cows showed LH peak at 50 h and two cows exhibit t he delayed LH peak at 58 and 82 h, respectively. It was observed that 100% cows responded positively to the CO-Synch+CIDR protocol (Table 1, Fig. 7 and 8).

Follicular and ovulatory characteristics: Highest growth rate per 24 h and the maximum diameter of dominant follicle was observed in Ovsynch group and lowest in CO-Synch+CIDR group but the difference was non-significant ($p>0.05$) among all the three treatment groups (Table 4). All cows ovulated after second GnRH in Ovsynch and CO-Synch+CIDR groups (100%) as compared to CIDR alone group (83.33%; $\chi^2 = 0.74$) but there was no significant difference ($p>0.05$) among these groups.

The mean ovulation time was earlier in Ovsynch treated cows (77.0±6.0 h) compared to CIDR alone and CO-Synch+CIDR groups (91.20±8.98 h and 79.20±2.93 h, respectively; Fig. 1a, 2a and 4a) with reference to the administration of $PGF_{2\alpha}$. Similarly, Ovsynch induced cows exhibited ovulation at 30.0±6.0 h with reference to administration of GnRH-2. Whereas in CO-Synch+CIDR ovulation occurred at 32.2±2.93 h but this difference was

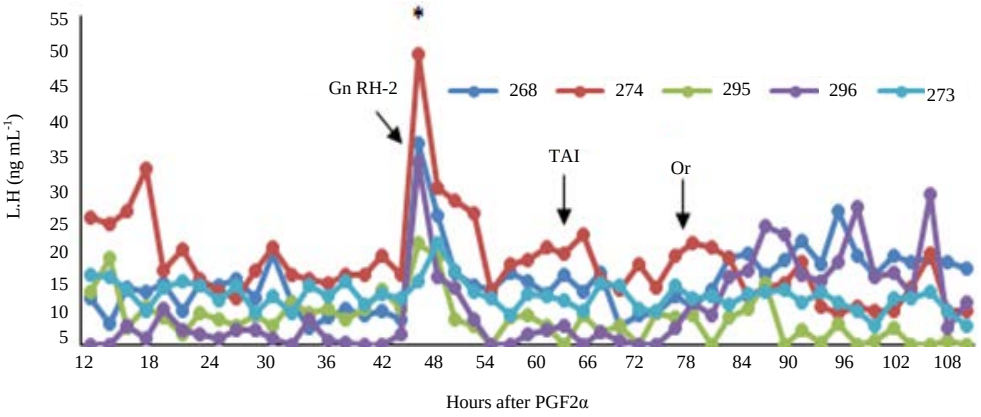

Fig. 1: The plasma LH concentration and Ovulation time (Ov) in Non-descript cows that responded to the Ovsynch protocol with synchrony LH peak. Blood sampling was done at 2 h intervals starting from 12-108 h post-PGF2a (*= LH peak); Ovsynch protocol

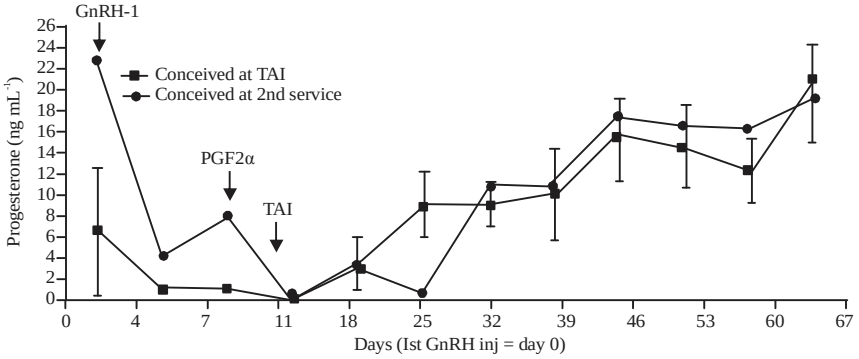

Fig. 2: The mean plasma P4 concentration in Non-descript cows (n = 5) that responded to the Ovsynch protocol indicated by P4 rise on day 11 post-PGF2a; Ovsynch protocol

Table 1: Interval between $PGF_{2\alpha}$ and LH peak, second GnRH and a LH peak, maximum concentration of LH peak, ovulatory response in Non-descript cows treated with the Ovsynch, CIDR alone and COSynch+CIDR protocols

Parameters	Ovsynch	CIDR	COSynch+CIDR
Number of Cows	5	6	7
Interval between $PGF_{2\alpha}$-LH (h)	48.40±0.40	71.67±9.74	54.86±4.71
Interval between 2nd GnRH-LH (h)	1.4±0.4	------	7.85±4.71
Peak LH conc. (ng mL^{-1})	29.40±5.87	36.01±4.87	26.19±3.67
Ovulatory response (%)	100	83.33	100

Table 2: Mean (±SE) follicular and ovulatory characteristics in Non-descript cows treated with different estrous synchronization protocols

Parameters	Ovsynch	CIDR alone	COSynch+CIDR	
Number of cows	5	6	7	p-values
Growth rate/day in mm (range)	1.43±0.15(1.0-1.67)	1.27±0.29(0.22-2.25)	1.06±0.13(0.63-1.46)	0.57
Diameter of DF in mm (range)	13.98±1.15(11.4-17.0)	12.20±0.39(11.5-13.6)	11.96±0.15(11.6-12.5)	0.09
Ovulation rate (%)	4/4 (100)	5/6 (83.33)	5/5 (100)	>0.05
Interval between $PGF_{2\alpha}$-LH (h)	48.40±0.40	71.67±9.74	54.86±4.71	0.06
Interval between $PGF_{2\alpha}$-OV (h)	77.0±6.0(60-84)	91.20±8.98(60-108)	79.20±2.93(72-84)	0.25
Interval between 2nd GnRH-OV (h)	30.0±6.0	----	32.2±2.93	0.73
Interval between LH-OV (h)	28.50±5.85	24.80±7.31	22.00±5.55	0.78
1st service conception rate	4(80) [a]	0(0.00) [b]	2(28.57) ab	p<0.05
2nd service conception rate	1(20)	5(83.33)	4(57.14)	p>0.05
Overall conception rate in 3 cycles	5(100)	5(83.33)	6(85.71)	p>0.05

a, b values with different superscript are significantly different at (p<0.05)

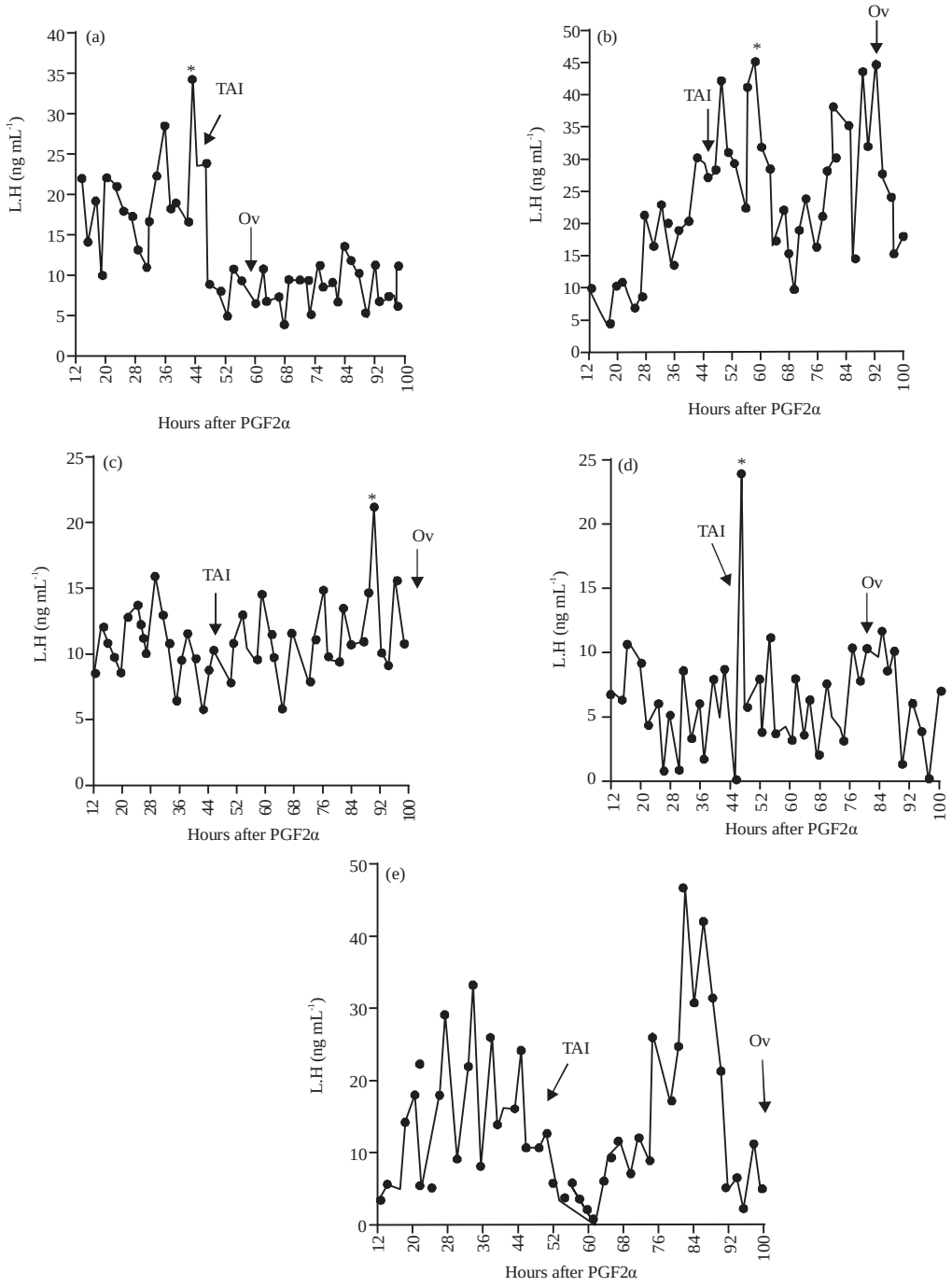

Fig. 3(a-e): The plasma LH concentration and Ovulation time (Ov) in Non-descript cows that responded to the CIDR alone protocol with non-synchronous LH peak. Blood sampling was done at 2 h intervals starting from 12-108 h post-PGF2a (*= LH peak) (a); 276 (b); 2881; (c) 282 (d) 283 and (e) 285

not significant ($p = 0.73$; $t_{(7)} = 0.35$) as shown in Table 2. The overall maximum conception rate (100%) was achieved by Ovsynch treatment as compared to CIDR alone and CO-Synch+CIDR treatments (83.33% and 85.71%, respectively). The conception rate at first service (80%) in the Ovsynch group was significantly higher ($p<0.05$) as compared to CIDR (0.00%) and CO-Synch+CIDR (28.57%) groups. Although, there was no

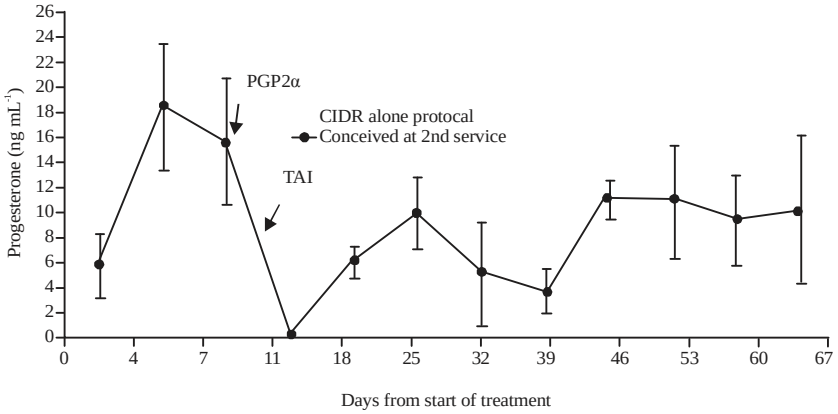

Fig. 4: The mean plasma P4concentration in Non-descript cows (n = 5) that responded to CIDR alone protocol indicated by P4 rise on day 11 post-PGF2a

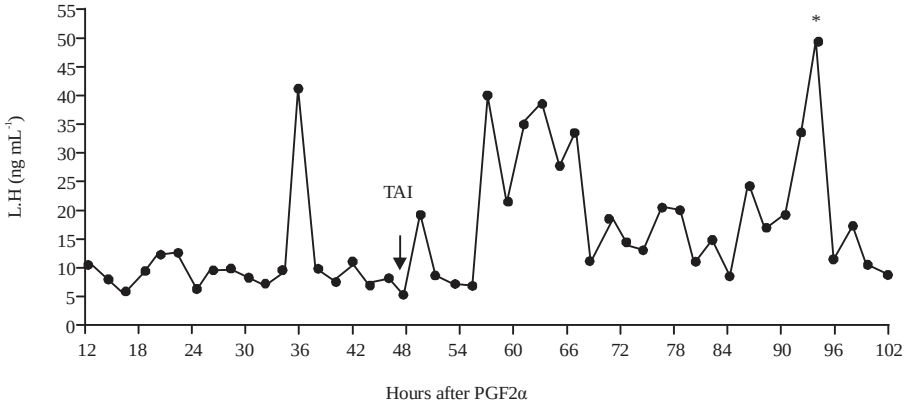

Fig. 5: The plasma LH concentrations in a Non-descript cow (Cow Id: 267) treated with CIDR alone protocol. The animal showed LH peak at 96 h after PGF2abut without increase in P4 following LH peak (*= LH peak)

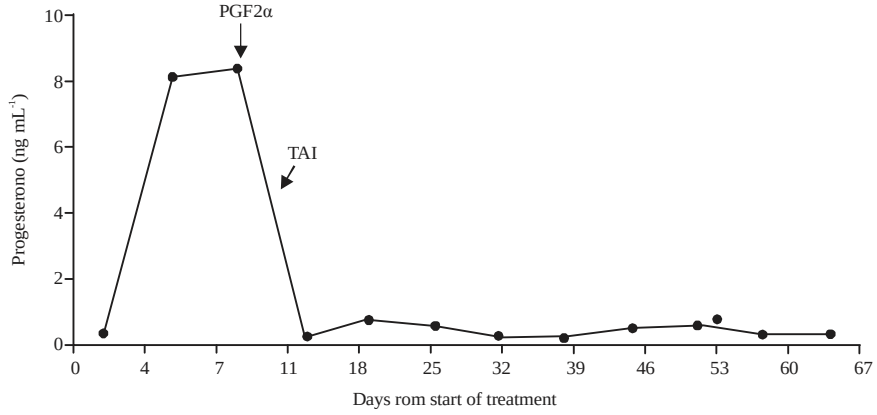

Fig. 6: Plasma progesterone concentrations in Non-descript cow number 267 treated with the CIDR alone protocol. The animal did not respond with ovulation as is evident from P4 levels. Blood sampling was done at 2 h intervals starting from 12-108 h post-PGF2a (* = LH peak)

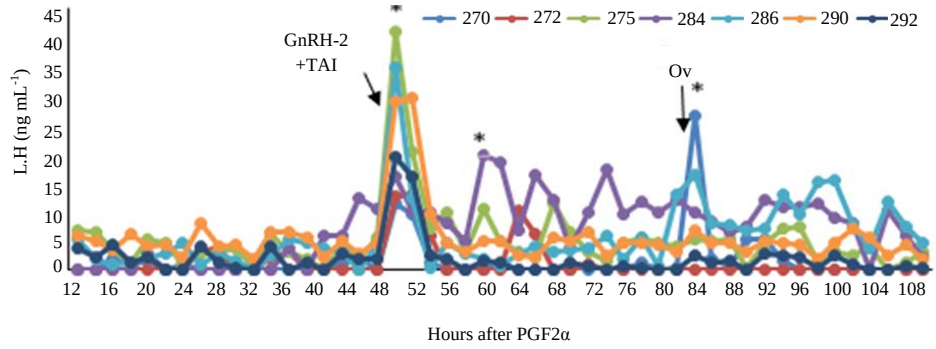

Fig. 7: The plasma LH concentration and Ovulation time (Ov) in Non-descript cows (n = 7) that responded to the CO-Synch+CIDR protocol with almost synchrony LH peak

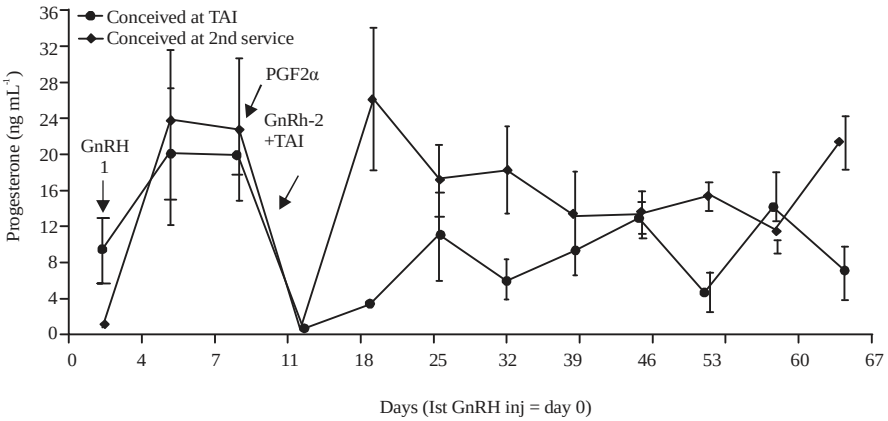

Fig. 8: The mean plasma P4concentration in Non-descript cows (n = 7) that responded to CO-Synch+CIDR protocol indicated by P4 rise on day 11 post-GnRH-1 and conceived at TAI

cow conceived at first service in CIDR group however up to 55.55% overall conception rate was achieved in second service (Table 2).

Present study has provided the comprehensive information on timing of ovulation in relation to the LH surge during induced estrus in Non-descript cows for the first time. Present findings of the LH peak at 48.40±0.40 h after $PGF_{2\alpha}$ administration with 29.40±5.87 ng mL^{-1} of LH concentration are in agreement with Monteiro *et al.*[21] who reported that the LH surge occurred at 46.7±4.9 h after $PGF_{2\alpha}$ administration with 27.2±5.7 ng mL^{-1} of LH concentration in Nelore cows (Bos indicus). A peak plasma LH concentration was clearly identified by 1-3 h post-GnRH in our study corresponds to those reported in Holstein cattle, i.e., 1-2 h[21]. A well synchronous LH surge within narrow intervals may be due to the administration of the second dose of GnRH (47 h) that increased the ovarian follicles growth resulting in greater amount of estradiol.

In agreement with previous findings[22] the cows treated with CIDR group in current study, showed a wide variation in intervals between administration of $PGF_{2\alpha}$ and preovulatory LH surge (44-96 h) . The delayed timing of LH surge with a wide variation in CIDR group was due to the reason that $PGF_{2\alpha}$ did not synchronize the stage of ovarian follicular development[23]. In CO-Synch+CIDR group, synchrony of LH surge was observed like Ovsynch group which might be due to the administration of second GnRH. The variation in the timing of pre-ovulatory surge of LH may be due to three different estrus induction treatments utilized in the experiments.

The concentrations of plasma P4 at the start of treatment, help in describing the reproductive and endocrine status of a cows and thus predict the possible outcome of the treatment. The raised plasma P4 level recorded on day 25 post-AI in different groups was due to the establishment of pregnancy. In present study, cows showed positive response to the Ovsynch and CO-Synch+CIDR protocols in term of synchronization of ovulation (100% vs. 83.33%, respectively) as confirmed by presence of CL on the ovary. The higher percentage of ovulatory response was due to exogenous GnRH

administration that directly stimulates the anterior pituitary to release of LH. Improving the ovulatory response to GnRH-1 results in better embryo quality and increased pregnancy per AI[24, 25]. Our findings are in contrary with Naikoo *et al*. [26] who reported that ovulatory estrus was induced in 66.66 and 83.33% of cows under Ovsynch and CIDR protocols, respectively. The low ovulatory response was reported with Ovsynch protocol by Keskin *et al*.[27] in cattle and the possible reasons for variation in results of different studies could be the stage of ovarian cycle at the beginning of the protocol, apart from variations in different environmental, management and genetic factors like nutritional status, parity, stage of lactation, suckling stimulus, season/climate, drug source, age, breed and species of animal[26].

The detailed knowledge about the ovarian follicles allowed the usage of hormones to regulate follicular growth and the beginning of ovulation, thus permit AI at a fix time without the need for detection of estrus in European (*Bos taurus*)[5] and Zebu breeds (*Bos indicus*)[11]. The follicular diameter and its growth rate in this study was not significantly different ($p>0.05$) in synchronized cows. Similar findings with Ovsynch treatment was observed in a previous study[28] in Sahiwal cows in which the size of pre-ovulatory follicles was 12.30±0.92 mm and mean growth rate of the dominant follicle (60 h post-PG injection) was 1.18±0.26 mm. Our finding also correlates with earlier study on *Bos indicus*-influenced cows[29]. The second dose of GnRH injection (24-48 h post-PGF_{2a}) causes a more accurate synchronization of ovulation in both *Bos taurus* and *Bos indicus* cattle[31] and permits timed artificial insemination[12]. Synchronous follicular wave emergence occurs only when treatment causes ovulation. Ovulation of large antral follicles is induced by the treatment of GnRH with a wave of new follicle emerging approximately 2 days later[30]. Also, it was observed that the ovulation rate with Ovsynch protocol was 75% in Nelore cows[31].

The determination of the time of ovulation help to inseminate the cows at a proper time after estrus induction. In present study the interval between $PGF_{2\alpha}$ and ovulation in CIDR and CO-Synch+CIDR groupswas similar to those reported in Holstein Gir crossbred cows (84.0±5.6 h)[32], Nelore cows (72.3±3.8 h)[21] and Zebu cows (68.8 h)[33]. The ovulation time in Ovsynch and CO-Synch+CIDR after the GnRH-2 administration was in agreement with Hassan *et al*.[28] who reported that ovulation time was 27±5.56 h in Sahiwal cows (*Bos indicus*). Similar results were described by Pursley *et al*.[5] that 100% Holstein cows and 75% of the heifers ovulated within 24-32 h after the administration of GnRH-2. The duration between the pre-ovulatory LH surge and ovulation with Ovsynch, CIDR and CO-Synch+CIDR was similar to those described for cows treated with $PGF_{2\alpha}$ with or without progestagen or estrogens treatments[23, 34]. The data suggests that ovulation may occur approximately 25 h after the LH surge, regardless of the hormonal treatments that initiate the preovulatory surge of LH.

CONCLUSION

The Ovsynch protocol induced a best synchrony of pre-ovulatory LH surge and ovulation. The time of LH peak in synchronized estrus did not differ from that in Bostaurus cows. The timing of LH peak can be considered to be a good indicator of the timing of ovulation to established fixed time AI in Non-descript cows. From this study, knowledge was obtained concerning the physiological events occurring during the Ovsynch, CIDR and CO-Synch+CIDR protocols for estrussynchronization which will be useful for improving FTAI program applied on Non-descript cows of AJ&K.

ACKNOWLEDGEMENT

The researcher extend their sincere thanks to the Livestock Development Research Center (LDRC) Muzaffarabad for use of their animals and facilities and National Center for Agriculture and Biology, Pakistan for laboratory analysis.

REFERENCES

01. Khan, M.I., S. Jalali, B. Shahid and S.A. Shami, 2014. Breeding efficiency of Indigenous×Jersey, Indigenous×Jersey×Friesian crossbred cows at livestock development research centre, Muzaffarabad, Azad Jammu and Kashmir. Proc. Pak. Acad. Sci., 51: 289-294.
02. Senthilkumar, K. and C. Chandrahasan, 2015. Oestrus behaviour in natural and induced estrus in dairy cattle by PGF2 a with GnRH and hCG. Int. J. Sci. Environ. Tech., 4: 243-247.
03. Zaituni Udin, Ferrdinal Rahim, Hendri , Yulia Yellita 2017. Effect of Ovsynch and Co-synch on Follicle Size and Conception Rate Indifferent Postpartum of Simmental Cows Asian J. Anim. Vet. Adv., 12: 115-122.
04. Malik, A., D.M. Heppy and N. Widaningsih, 2018. Comparing response of estrus synchronisation in the heifers and cows on estrus initial, duration of estrus and pregnancy rate bali cattle. Adv. Anim. Vet. Sci., 6: 242-245.
05. Pursley, J.R., M.O. Mee and M.C. Wiltbank, 1995. Synchronization of ovulation in dairy cows using PGF2a and GnRH. Theriogenology, 44: 915-923.
06. Hansel, W., 1961. Estrous cycle and ovulation control in cattle. J. Dairy Sci., 44: 2307-2314.

07. Lamb, G.C., J.S. Stevenson, D.J. Kesler, H.A. Garverick, D.R. Brown and B.E. Salfen, 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2a for ovulation control in postpartum suckled beef cows. J. Anim. Sci., 79: 2253-2259.
08. Pennington, A.J., 2013. Heat detection in dairy cattle. University of Arkansas, Fayetteville, Arkansas.
09. Mondal, M., C. Rajkhowa and B.S. Prakash, 2006. Oestrous behaviour and timing of ovulation in relation to onset of oestrus and LH peak in Mithun (Bos frontalis) cows. Reprod. Domestic Anim., 41: 479-484.
10. Ginther, O.J., J.P. Kastelic and L. Knopf, 2003. Composition and characteristics of follicular waves during the bovine estrous cycle. Anim. Reprod. Sci., 20: 187-200.
11. Baruselli, P.S., E.L. Reis, M.O. Marques, L.F. Nasser and G.A. Bo, 2004. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. Anim. Reprod. Sci., 82: 479-486.
12. Pursley, J.R., M.R. Kosorok and M.C. Wiltbank, 1997. Reproductive management of lactating dairy cows using synchronization of ovulation. J. Dairy Sci., 80: 301-306.
13. Macmillan, K.L., 2010. Recent advances in the synchronization of estrus and ovulation in dairy cows. J. Reprod. Dev., 56: S42-S47.
14. Stevenson, J.S., S.L. Pulley and H.I. Mellieon Jr, 2012. Prostaglandin F2a and gonadotropin-releasing hormone administration improve progesterone status, luteal number and proportion of ovular and anovular dairy cows with corpora lutea before a timed artificial insemination program. J. Dairy Sci., 95: 1831-1844.
15. Yizengaw, L., 2017. Review on estrus synchronization and its application in cattle. Int. J. Adv. Res. Biol. Sci., 4: 67-76.
16. Shahid, B., M.I. Khan, S.M.H. Andrabi, A. Razzaq and M.N. Khan, 2019. Estrus duration and expression in natural and induced estrus in indigenous (Bos indicus) cattle of Azad Jammu and Kashmir. J. Anim. Plant Sci., Vol. 29, No. 3.
17. Wildman, E.E., G.M. Jones, P.E. Wagner, R.L. Boman, H.F. Troutt Jr. and T.N. Lesch, 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci., 65: 495-501.
18. Kanai, Y. and N. Ischikawa, 1988. Pulsatile secretion of luteininzing hormone and plasma levels of ovarian steroids during the estrous cycle in the Shiba goat. Japan J. Anim. Reprod., 34: 105-110.
19. Pulley, S.L., D.H. Keisler and J.S. Stevenson, 2015. Concentrations of luteinizing hormone and ovulatory responses in dairy cows before timed artificial insemination. J. Dairy Sci., 98: 1-14.
20. Gordon, M.B., N. Dinn and R. Rajamahendran, 2010. Effects of presynchronization and postinsemination treatments on pregnancy rates to a timed breeding Ovsynch protocol in dairy cows and heifers. Can. J. Anim. Sci., 90: 35-44.
21. Picard-Hagen, N., G. Lhermie, S. Florentin, D. Merle, P. Frein and V. Gayrard, 2015. Effect of gonadorelin, lecirelin and buserelin on LH surge, ovulation and progesterone in cattle. Theriogenology, 84: 177-183.
22. Evans, A.C.O., P. O'keeffe, M. Mihm, J.F. Roche, K.L. Macmillan and M.P. Boland, 2003. Effect of oestradiol benzoate given after prostaglandin at two stages of follicle wave development on oestrus synchronisation, the LH surge and ovulation in heifers. Anim. Reprod. Sci., 76: 13-23.
23. Monteiro, F.M., D.S. Meloa, M.M.G. Ferreira, L.M. Carvalho and E. Sartoreli e Sartoreli et al., 2009. LH surge in Nelore cows (Bos indicus), after induced estrus or after ovarian superestimulation. Anim. Reprod. Sci., 110: 128-138.
24. Cerri, R.L., H.M. Rutigliano, R.G.S. Bruno and J.E. Santos, 2008. Progesterone concentration, follicular development and induction of cyclicity in dairy cows receiving intravaginal progesterone inserts. Anim. Reprod. Sci., 110: 56-70.
25. Rutigliano, H.M., F.S. Lima, R.L.A. Cerri, L.F. Greco and J.M. Vilela et al., 2008. Effects of method of presynchronization and source of selenium on uterine health and reproduction in dairy cows. J. Dairy Sci., 91: 3323-3336.
26. Naikoo, M., A.J. Dhami and A. Ramakrishnan, 2016. Effect of estrus synchronization on plasma progesterone profile and fertility response in postpartum suckled anestrous Kankrej cows. Indian J. Anim. Res., 50: 460-465.
27. Keskin, A., G. Yilmazbas-Mecitoglu, A. Gumen, E. Karakaya, Y. Celik, H. Okut and M.C. Wiltbank, 2011. Comparison of responses to ovsynch between Holstein-Friesian and Swedish red cows. J. Dairy Sci., 94: 1784-1789.28.
28. Hassan, M., A. Husnain, M.I. Naveed, U. Riaz and N. Ahmad, 2016. Effect of ovsynch versus prostaglandin F2a protocol on estrus response, ovulation rate, timing of ovulation and pregnancy per artificial insemination in Sahiwal cows. Anim. Sci. J., 88: 445-450.

29. Saldarriaga, J.P., D.A. Cooper, J.A. Cartmill, J.F. Zuluaga, R.L. Stanko and G.L. Williams, 2007. Ovarian, hormonal and reproductive events associated with synchronization of ovulation and timed appointment breeding of Bos indicus-influenced cattle using intravaginal progesterone, gonadotropin-releasing hormone and prostaglandin F2a. J. Anim. Sci., 85: 151-162.
30. Twagiramungu, H., L.A. Guilbault and J.J. Dufour, 1995. Synchronization of ovarian follicular waves with a gonadotropin-releasing hormone agonist to increase the precision of estrus in cattle: A review. J. Anim. Sci., 73: 3141-3151.
31. Barros, C.M., M.B.P. Moreira, R.A. Figueiredo, A.B. Teixeira and L.A. Trinca, 2000. Synchronization of ovulation in beef cows (Bos indicus) using GnRH, PGF2a and estradiol benzoate. Theriogenology, 53: 1121-1134.
32. Pfeifer, L.F.M., L.G.B. Siqueira, E.K.N. Arashiro, N.A.D. Castro and J.H.M. Viana, 2016. Prostaglandin F2a or estradiol benzoate to induce ovulation in timed artificially inseminated dairy cows. Pesquisa Agropecuaria Brasileira, 51: 738-744.
33. Cavalieri, J., I. Rubio, J.E. Kinder, K.W. Entwistle and L.A. Fitzpatrick, 1997. Synchronization of estrus and ovulation and associated endocrine changes in bos indicus cows. Theriogenol., 47: 801-814.
34. Hanlon, D.W., N.B. Williamson, J.J. Wichtel, I.J. Steffert, A.L. Craigie and D.U. Pfeiffer, 2002. Ovulatory responses and plasma luteinizing hormone concentrations in dairy heifers after treatment with exogenous progesterone and estradiol benzoate. Theriogenology, 47: 963-975.