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POST-PARTUM SUB-CLINICAL ENDOMETRITIS- ALTERATION IN UTERINE HAEMODYNAMICS, INFLAMMATORY MARKERS ON TREATMENT WITH MODIFIED ESTRUS SYNCHRONIZATION PROTOCOLS IN DAIRY COWS

Endometritis subclínica posparto - Alteración de la hemodinámica uterina, marcadores inflamatorios en el tratamiento con protocolos modificados de sincronización del estro en vacas lecheras

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ABSTRACT

Forty-one dairy cows (n=41) were enrolled to envisage the changes in uterine haemodynamics during sub-clinical endometritis (SCE) and its treatment with modified estrus synchronization protocols at 8 weeks post-partum. Trans-rectal Doppler sonography of both the middle uterine arteries (MUAs) was carried out for assessment of uterine perfusion whereas serum inflammatory markers i.e. IL-6 and C-RP were measured at 8 weeks post-partum and estrus (induced or spontaneous). Modified estrus synchronization protocols (MG6G and MG6GP) were used to adjudge their efficacy in post-partum dairy cows diagnosed with SCE and reproductive parameters were recorded. As a part of result, Doppler indices of both the MUAs at estrus i.e. TAMEAN, TAMAX, Blood flow volume-TAMEAN and TAMAX and diameter of MUA, were significantly lower ($P<0.05$) after application of MG6G and MG6GP protocols in SCEP as compared to SCEP control cows. Similarly, the IL-6, C-RP concentrations and PMNCs proportion (%) were significantly higher ($P<0.05$) in SCEP control as compared to cows treated with MG6G and MG6GP protocols. Moreover, on the day of estrus, the uterine haemodynamics, concentration of serum inflammatory markers and PMNCs proportion (%) in treated cows was at par with SCE negative control (SCEN) cows. In terms of reproductive performance, days open were recorded to be significantly lower ($P<0.01$) in treated and SCEN group as compared to SCEP control cows. In conclusion, sub-clinical endometritis led higher uterine perfusion, release of pro-inflammatory cytokines and PMNCs proportion which happened to plummet the post-partum reproductive performance was successfully managed with modified estrus synchronization protocols.

Keywords: Dairy cows; inflammatory markers; sub-clinical endometritis; uterine haemodynamics

RESUMEN

Se revisaron vacas lecheras (n = 41) para prever los cambios en la hemodinámica uterina durante la endometritis subclínica (SCE) y su tratamiento con protocolos modificados de sincronización del estro a 8 semanas posparto. Se realizaron una ecografía Doppler transrectal de ambas arterias uterinas medias (MUA) para evaluar la perfusión uterina, mientras que los marcadores inflamatorios séricos, es decir, IL-6 y C-RP, se midieron a las 8 semanas después del parto y el estro (inducido o espontáneo). Se utilizaron protocolos de sincronización de celo modificados (MG6G y MG6GP) para determinar su eficacia en vacas lecheras posparto diagnosticadas con SCE y se registraron los parámetros reproductivos. Como parte del resultado, los índices Doppler de ambos MUA en el estro, es decir, TAMEAN, TAMAX, volumen de flujo sanguíneo-TAMEAN y TAMAX y diámetro de MUA, fueron significativamente más bajos ($P <0.05$) después de la aplicación de los protocolos MG6G y MG6GP en SCEP en comparación a las vacas de control de SCEP. De manera similar, las concentraciones de IL-6, C-RP y la proporción de PMNC (%) fueron significativamente más altas ($P <0.05$) en el control SCEP en comparación con las vacas tratadas con los protocolos MG6G y MG6GP. Además, en el día del estro, la hemodinámica uterina, la concentración de marcadores inflamatorios séricos y la proporción de PMNC (%) en las vacas tratadas estaban a la par con las vacas de control negativo SCE (SCEN). En términos de performance reproductiva, se observó que los días abiertos fueron significativamente

más bajos (P <0.01) en el grupo tratado y grupo SCEN en comparación con las vacas control (SCEP). En conclusión, la endometritis subclínica condujo a una mayor perfusión uterina, la liberación de citocinas proinflamatorias y la proporción de PMNC que redujo el rendimiento reproductivo posparto y se manejó con éxito con los protocolos modificados de sincronización del estro.

Palabras clave: Vacas lecheras; marcadores inflamatorios; endometritis subclínica; hemodinámica uterina

INTRODUCCION

Unavoidable contamination of uterus during parturition often leads to persistence of infection i.e. sub-clinical endometritis (SCE), which affects the survival of an embryo (Gilbert, 2011) due to two reasons: a) toxic effect of bacterial compounds; b) reduced uterine secretions (McDougall, 2001), therefore, reversal of inflammatory changes is required via enhancement of uterine defense mechanism (Sheldon et al., 2008). Many researchers have assessed uterine inflammation via endometrial cytology (Pascottini et al., 2016), uterine perfusion (Sharma et al., 2019) and measurement of serum inflammatory markers (Nehru et al., 2019; Elsayed et al., 2020). Different treatment options for SCE include administration of prostaglandins alone i.e. PGF2α (Galvao et al., 2009; Haimerl et al., 2013) and PGF2α along with estrus synchronization protocols (presynch protocols; Coto, 2016), intra-uterine antibiotics (Denis-Robichaud and Dubuc, 2015), non-steroidal anti-inflammatory drugs (NSAIDs; Priest, 2013; Pascottini et al., 2020) and Samia treat (SAT) i.e. boiling water (El-Rheem et al., 2019). Thus, the current study was carried out with two objectives; (i) assessment of alteration in uterine haemodynamics as well as serum inflammatory markers; (ii) efficacy of modified estrus synchronization protocols with regard to reproductive efficiency in dairy cows diagnosed with post-partum sub-clinical endometritis.

MATERIALS AND METHODS

Animals

Forty-one healthy Jersey crossbred multiparous cows (Parity 3-4; N=41) having no previous history of any clinico-reproductive disorders i.e. dystocia, retention of placenta, metritis, ketosis and mastitis, reared in a loose housing system under standard management conditions, fed a total mixed ration, once daily ad libidum, and had unrestricted access to water in university dairy farm were enrolled for the study after normal parturition. The cows have not received any treatment during the pre-partum period and course of study and at calving, their health status was assessed on the basis of normal rectal temperature (38.67±0.02°C). Cows were milked twice daily (04:00 and 15:00 h). All the experiments have been carried after the approval of ethical committee of the Dr. G.C. Negi College of Veterinary and Animal Sciences, CSKHPKV, Palampur.

Endometrial cytology

Cytotape method of endometrial cytology was employed for adjudging the polymorphonuclear cells (PMNCs) proportion for diagnosis of sub-clinical endometritis at 8 weeks post-partum. Cytotape assembly was introduced into the vagina after cleaning the vulval area and sheath was perforated at external os of the cervix followed by introduction of steel rod rolled with paper tape into the body of the uterus. Sample was taken by rolling the rod having tape, on the wall of uterine body with gentle pressure of index finger through rectum. The cytotape was then retracted from the uterus and smear was formed by gently rolling the tape on clean glass slide. Prepared slides were air dried, fixed in methanol for 15 minutes and then stained with modified Wright-Giemsa stain for 45 minutes. All the slides were evaluated by optical light microscope and cells were counted in a total of 10 fields and the percentage of epithelial cells, endometrial cells and PMNCs were assessed at 40X magnification (Rana et al., 2020). Based on these findings, the cows were divided into sub-clinical endometritis positive (SCEP; n=27) i.e. PMNCs percentage ≥5% (Pascottini et al., 2016) and sub-clinical endometritis negative (SCEN; n=14) groups (Figure 1a, 1b).

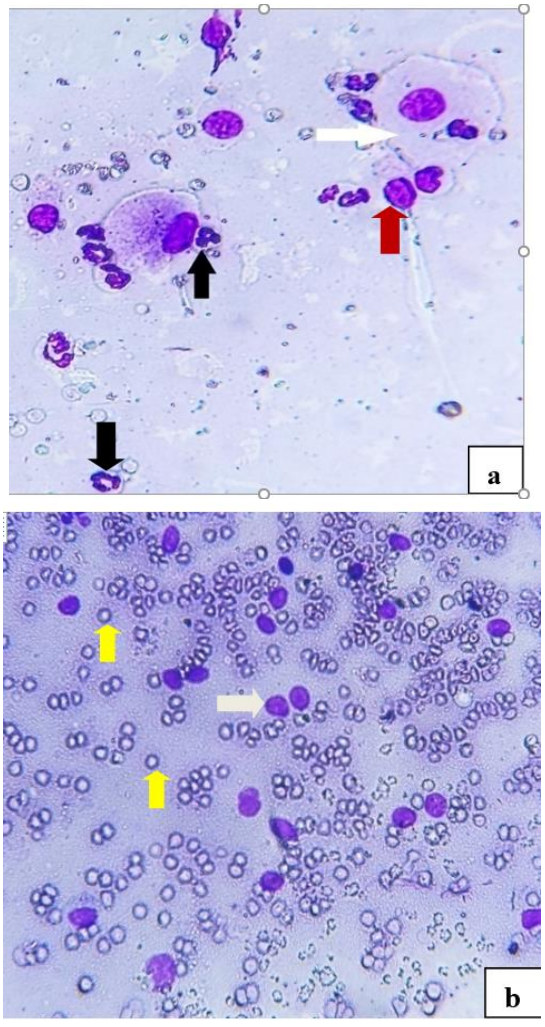

Figure 1. a) Endometrial cytology of uterus in SCEP dairy cows (Wright Giemsa stain): (a) epithelial cell (white arrow), endometrial cell (red arrow) & PMNCs (black arrow) - before treatment (40X). b) Endometrial cytology of uterus in SCEP dairy cows (Wright Giemsa stain): endometrial cell (grey arrow) & RBCs (yellow arrow)- after treatment (40X)- RBCs number has increased due to sample collection on the day of induced estrus.

Trans-rectal Doppler sonography of middle uterine artery for assessment of uterine perfusion

For monitoring of uterine blood flow, Doppler examination of middle uterine artery (MUA) ipsilateral and contralateral to ovarian dominant follicle was performed at 8 weeks post-partum and estrus (treated and non-treated cows), using a linear probe of portable Mindray Z5 ultrasound machine at a frequency of 7.5 MHz, with a filter of 100 Hz and Doppler angle varying between 30° and 60°. The haemodynamic indices displayed for each waveform by applying the automatic mode were recorded. The MUA's transverse diameter (D) was calculated from the mean of three measurements of the diameter made from frozen two dimensional grey scale images just before Doppler measurements.

Uterine blood flow volume in mL/min was calculated using the equation (Varughese et al., 2013):

- Blood flow volume-TAMEAN = TAMEAN × π × (D×0.1/2)2 × 60
- Blood flow volume-TAMAX = TAMAX × π × (D×0.1/2)2 × 60

Inflammatory markers assay

Blood samples (n=82) from forty one cows were collected at 8 weeks post-partum and estrus (treated and non-treated) followed by separation of serum by slant method and stored at -20°C for pending analysis. Inflammatory markers i.e. Interleukin-6 (IL-6) and C-Reactive protein (C-RP) estimation was done from the stored serum samples (-20°C) after thawing at room temperature. ELISA kits were used to analyze IL-6 (Sensitivity-10.50ng/L) and C-RP (Sensitivity-0.24mg/L) via TECAN SUNRISE Microplate Absorbance Reader (TECAN Austria GmbH, Austria). Repeated thawing of serum samples was strictly avoided.

Modified estrus synchronization protocols for treatment of sub-clinical endometritis

Modified estrus synchronization protocols (MG6G and MG6GP) have been used to judge their efficacy in post-partum dairy cows diagnosed with sub-clinical endometritis. Modified estrus synchronization protocols used are given below:

MG6G protocol

In cows diagnosed with sub-clinical endometritis, administration of 500 µg PGF2α (Pragma®; Intas Pharamceuticals Ltd.) was done on day -4 i.e. day 57 after parturition followed by 10 µg GnRH (Receptal®; MSD Animal Health India Ltd.). On day 6, 10 µg GnRH (Receptal®; MSD Animal Health India Ltd.) was administered again which was followed by 500 µg PGF2α (Pragma®; Intas Pharamceuticals Ltd.) on day 13. At last, 10 µg GnRH (Receptal®; MSD Animal Health India Ltd.) was administered on day 15. All the hormonal preparations were administered via intra-muscular route. Fixed time artificial insemination (FTAI) was done at 22-24 hours after the last GnRH administration and followed by repeat AI.

MG6GP protocol

In cows diagnosed with sub-clinical endometritis, administration of 500 µg PGF2α (Pragma®; Intas Pharamceuticals Ltd.) was done on day -4 i.e. day 57 after parturition followed by 10 µg GnRH (Receptal®; MSD Animal Health India Ltd.). On day 6, 10 µg GnRH (Receptal®; MSD Animal Health India Ltd.) was administered again which was followed by 500 µg PGF2α (Pragma®; Intas Pharamceuticals Ltd.) on day 13 and 15. At last, 10 µg GnRH (Receptal®; MSD Animal Health India Ltd.) was administered on day 15. All the hormonal preparations were administered via intra-muscular route. Fixed time artificial insemination (FTAI) was done at 22-24 hours after the last GnRH administration and followed by repeat A.I.

Pre-ovulatory follicle at the time of A.I.

The effect of treatment on ovarian follicular growth was monitored in treatment and non-treatment groups with the aid of trans-rectal ultrasonography by linear rectal probe at 7.5 MHz frequency. FTAI was done after visualizing the pre-ovulatory follicle on the day of estrus (spontaneous and induced).

Monitoring of post-partum reproductive parameters

Following parturition, reproductive parameters i.e. days to first artificial insemination (A.I.), number of inseminations per conception and days open were also recorded in treated, SCEP and SCEN control cows.

Statistical analysis

The obtained data was statistically analyzed using repeated measures ANOVA, Student's t-test for testing the significance of parameters with NCSS 2020, USA (Version 20.0.1).

RESULTS

Haemodynamic parameters of MUA and PMNCs proportion (%) pre- and post-application of MG6G and MG6GP protocols

MG6GP modified estrus synchronization protocol led to a significant increase ($p<0.05$) in RI on the day of induced estrus as compared to day 56 post-partum, however, the difference was non-significant ($P>0.05$) in MG6G, SCEP and SCEN control group. Similarly, the velocity of blood flow to uterus i.e. TAMEAN and TAMAX, had a significant decrease ($P<0.01$) following treatment with MG6G and MG6GP modified estrus synchronization protocols as compared to SCEP control cows. However, the blood flow volume to the uterus i.e. BFV-TAMEAN and BFV-TAMAX (Figure 2a, 2b, 2c and 2d), reduced significantly following treatment in MG6G and MG6GP ($p<0.01$) and no treatment ($P<0.05$) in SCEP control group. The diameter of middle uterine artery also decreased significantly ($P<0.01$ & 0.05) in treatment and non-treatment groups (SCEP and SCEN control) on day 56 and day of induced and spontaneous estrus. However, the DPD and AT had no significant difference ($p>0.05$) in their values following treatment and no treatment in SCEP dairy cows. Similarly, PMNCs proportion reduced more significantly ($P<0.01$) in treatment groups as compared to SCEP control non-treatment group (Table 1).

Serum inflammatory markers pre- and post-application of MG6G and MG6GP protocols

In present study, a significant decrease in IL-6 levels was recorded on the day of induced estrus after application of modified estrus synchronization protocols i.e. MG6G ($P<0.05$) and MG6GP ($P<0.01$), on day 56 post-partum as compared to SCEP control group at spontaneous estrus. C-Reactive protein, on the other hand, had a significant decrease in MG6GP group ($P<0.01$) when compared to MG6G group (Table 1) on the day of induced estrus.

Table 1. Comparative adjudgment of uterine inflammation based on uterine perfusion, PMNCs proportion (%) and serum inflammatory markers in SCEP and SCEN dairy cows (n=41) treated and not treated with modified estrus synchronization protocols at 8 weeks post-partum (Mean±S.E.).

Parameters	Days	Treatment groups			
		MG6G (n=10)	MG6GP (n=10)	SCEP control (n=7)	SCEN control (n=14)
Pulsatility index	Day 56	1.14±0.06	1.08±0.06	1.12±0.09	1.32±0.15
	On day of estrus	1.18±0.08	1.16±0.10	1.15±0.12	1.36±0.12
Resistance index	Day 56	0.63±0.02	0.58±0.03^{y}	0.62±0.04	0.65±0.04
	On day of estrus	0.66±0.03	0.68±0.02^{x}	0.64±0.05	0.68±0.03
TAMEAN (cm/sec)	Day 56	24.35±1.18^{a}	26.63±1.20^{a}	25.85±1.43	19.34±1.38
	On day of estrus	18.15±0.55^{bY}	18.12±0.72^{bY}	22.42±1.32^{X}	18.95±0.57^{Y}
TAMAX (cm/sec)	Day 56	45.96±1.89^{a}	48.48±1.95^{a}	45.58±2.46	36.36±2.28
	On day of estrus	34.19±1.58^{bY}	34.16±1.53^{bY}	40.36±1.88^{X}	35.20±1.26^{Y}
Diameter of the MUA (mm)	Day 56	8.45±0.01^{a}	8.57±0.01^{a}	8.58±0.04^{x}	8.41±0.03^{a}
	On day of estrus	7.95±0.01^{bB}	7.90±0.02^{bB}	8.32±0.03^{yA}	7.98±0.02^{bB}
Blood flow volume-TAMEAN (mL/min)	Day 56	830.56±47.12^{a}	870.40±50.70^{a}	864.43±55.72^{x}	654.06±52.05
	On day of estrus	545.97±39.70^{b}	541.48±45.23^{b}	680.21±52.32^{y}	596.76±28.34
Blood flow volume-TAMAX (mL/min)	Day 56	1510.49±76.40^{a}	1602.94±77.92^{a}	1556.04±86.64^{x}	1240.60±92.79
	On day of estrus	999.29±67.53^{bY}	983.39±71.03^{bY}	1198.50±79.82^{yX}	1032.44±56.33
Doppler pulse duration (msec)	Day 56	718.18±19.50	745.90±22.64	735.52±27.43	683.72±36.86
	On day of estrus	680.42±15.62	684.50±16.49	720.08±24.95	692.46±14.26
Acceleration time (msec)	Day 56	152.78±10.28	141.64±10.80	145.46±14.08	154.03±15.43
	On day of estrus	158.12±9.76	154.30±9.22	151.58±12.34	159.32±8.74
Interleukin-6 (ng/L)	Day 56	569.20±75.62^{x}	578.17±37.09^{a}	609.50±78.92	350.69±35.62
	On day of estrus	316.16±50.81^{yY}	270.36±22.86^{bY}	404.89±36.19^{X}	310.44±28.74
C-Reactive protein (mg/L)	Day 56	20.03±1.08	20.09±1.18^{a}	21.88±1.13	16.38±0.86
	On day of estrus	16.65±1.85^{B}	13.60±1.85^{bB}	19.82±0.53^{A}	15.32±0.85^{B}
Polymorphonuclear cells (%)	Day 56	5.00±0.26^{a}	5.88±0.39^{a}	6.45±0.69	Nil
	On day of estrus	1.90±0.53^{bB}	0.80±0.42^{bB}	4.49±0.55^{A}	Nil

a,b (P<0.01) and x,y (P<0.05) Values with different superscripts within the same column for the same parameter and category are significantly different

A,B (P<0.01) and X,Y (P<0.05) Values with different superscripts within the same row for the same parameter and day are significantly different

Table 2. Pre-ovulatory follicle diameter (mm) and post-partum reproductive performance in SCEP and SCEN dairy cows (n=41) treated and not treated with modified estrus synchronization protocols (Mean±S.E.)

Treatment groups	Parameters				
	Pre-ovulatory follicle diameter (mm)	First insemination conception rate (%)	Days to first artificial insemination	Number of inseminations per conception	Days open
MG6G (n=10)	11.45±0.38^{a}	30.00	76	1.80±0.49	113.80±7.29^{by}
MG6GP (n=10)	12.93±0.26^{ax}	70.00	77	1.56±0.29	109.67±4.17^{by}
SCEP control (n=7)	8.98±0.48^{b}	14.29	117.67±4.39^{x}	2.33±0.57	166.67±4.24^{a}
SCEN control (n=14)	10.17±0.27^{y}	21.43	102.73±1.73^{y}	1.91±0.21	133.64±2.96^{bx}

a,b Values with different superscripts within the same column and parameter differ significantly (P<0.01)

x,y Values with different superscripts within the same column and parameter differ significantly (P<0.05)

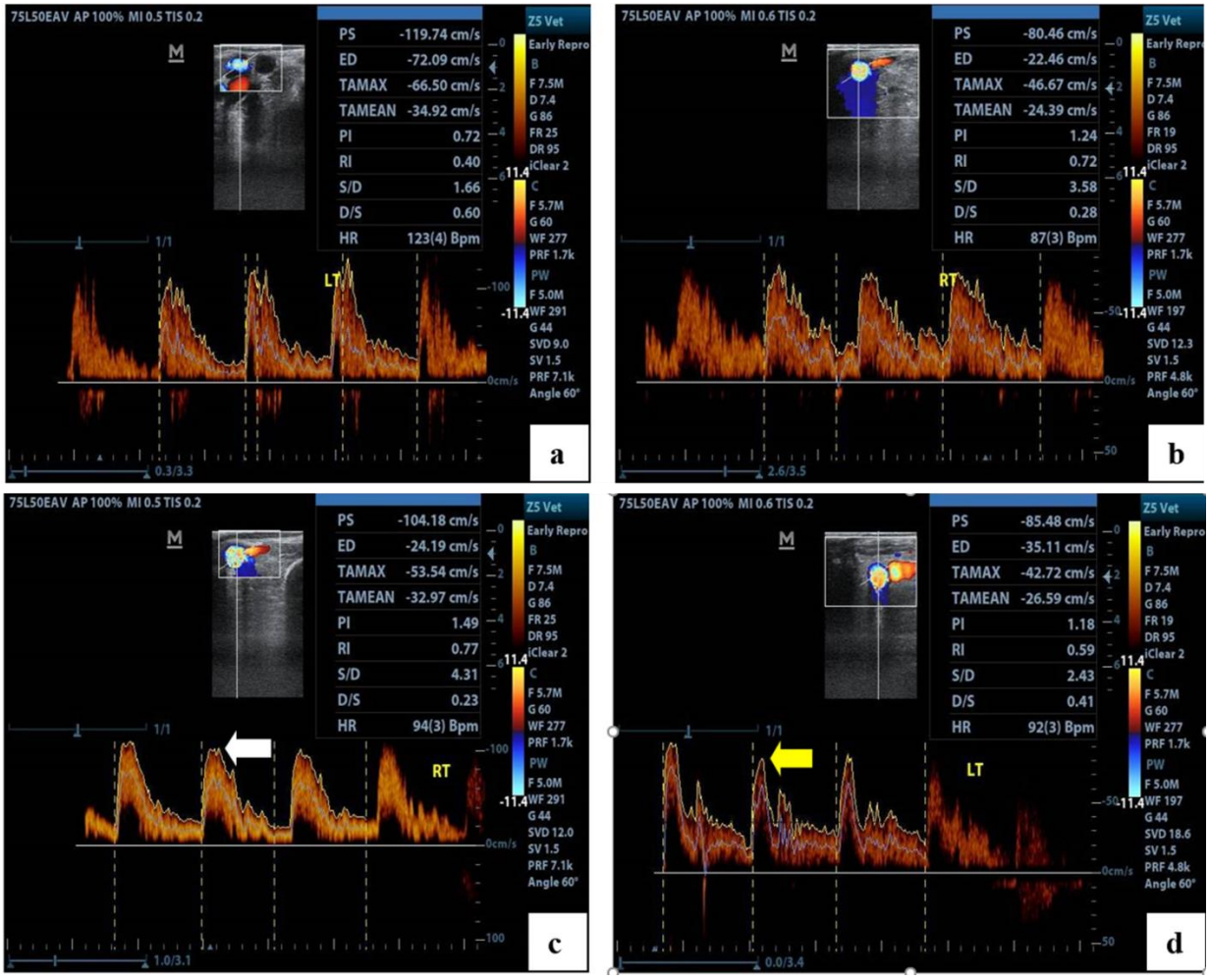

Figure 2. a) Spectral Doppler waveform of MUA ipsilateral to dominant follicle in an SCEP dairy cow treated with modified estrus synchronization protocols- before treatment i.e. day 56 post-partum, characterized by low PI, RI and high TAMEAN, TAMAX which is evident of MUA vasodilation due to uterine inflammation. b) After treatment i.e. at FTAI- MUA waveform characterized by increased PI, RI and decreased TAMEAN, TAMAX which is evident of less MUA vasodilation due to decrease in uterine inflammation. c) Spectral Doppler waveform of MUA contralateral to dominant follicle in an SCEP dairy cow treated with modified estrus synchronization protocols- before treatment i.e. day 56 post-partum, characterized by broader systolic peak (white arrow) and high TAMEAN, TAMAX which is evident of MUA vasodilation due to uterine inflammation y d) After treatment i.e. day 76 post-partum- MUA waveform characterized by narrower systolic peak (yellow arrow) and decreased TAMEAN, TAMAX which is evident of less MUA vasodilation due to decrease in uterine inflammation

Pre-ovulatory follicle size and impact on reproductive performance

A significantly higher ($p<0.01$ & 0.05) pre-ovulatory follicle diameter in cows treated with MG6G and MG6GP protocols was present at induced estrus as compared to SCEP and SCEN control cows at spontaneous estrus. The post-partum reproductive performance in terms of days to first AI and days open were significantly higher ($p<0.01$) in SCEP as compared to SCEN and MG6G and MG6GP cows. Similarly, the first insemination conception rate (%) was comparatively higher in MG6GP treatment group as compared to SCEP and SCEN control group (Table 2).

DISCUSSION

Any impairment in immune function during peri-partum period results in persistent uterine inflammation and sub-optimal reproductive performance (Patra et al., 2013; Sahadev et al., 2019). Doppler mode of ultrasonography still remains an unexplored tool for diagnosis of endometritis in dairy cows (Arias et al., 2018). During post-partum period, endometrial regeneration requires a lot of blood supply (upto 6 weeks post-partum; Sheldon and Dobson, 2004) whereas increased volume of blood flow to uterus beyond that period is mainly due to nitric oxide induced vasodilation of MUA under the influence of endometrial inflammation (Krueger et al., 2009; Pancarci et al., 2012). The findings of haemodynamic indices in present study were in concurrence with the results reported by Debertolis et al. (2016), Sharma et al. (2019) and Sharma et al. (2021). However, DPD and AT did not hold any significance ($P>0.05$) as haemodynamic indices in assessment of post-partum uterine inflammation and was also in concurrence with Sharma et al. (2019).

For treatment of post-partum SCE, the administration of PGF2α alone has been tried by various researchers (Galvao et al.,

2009; Dubuc et al., 2011) probably due to uterotonic effect, amelioration of uterine defense mechanism and decreasing the PMNCs proportion (%) (Lewis, 2004; Kasimanickam et al., 2005). The treatment of sub-clinical endometritis via administration of PGF2α in conjunction with estrus synchronization protocols has been adjudged in terms of reproductive performance (Galvao et al., 2009; Lima et al., 2013) although its efficacy in reducing the uterine perfusion and of SCEP cows is not known yet and need to be studied further.

A robust but well regulated inflammatory response i.e. regulation of pro-inflammatory cytokines, is very much needed for restoration of ovarian function following elimination of uterine pathogens (Sheldon et al., 2018) but prolonged uterine response to inflammatory agents beyond 5-6 weeks may predispose cows to poor post-partum reproductive performance via impairment of steroidogenesis and ovulation process (Cheong et al., 2017; Stassi et al., 2017; Pascottini et al., 2020).

Similar to present study, Gabler et al. (2010), Fischer et al. (2010), Kim et al. (2014), Brodzki et al. (2015) and Elsayed et al. (2020) have also reported higher serum concentrations of IL-6 and C-RP at 7-8 weeks post-partum in cows diagnosed with SCE when compared with cows having no uterine inflammation. However, no literature regarding adjudging the efficacy of modified estrus synchronization protocols for balanced regulation of IL-6 and C-RP levels and optimal reproductive performance has been documented yet.

The ovulatory capacity of bovine follicles generally occur at a size >10 mm (Sartori et al., 2001) as higher competence and better RNA synthesis was mainly observed in large sized follicles (Fair et al., 1995; Green et al., 2011). In present study, the cows treated with modified estrus synchronization protocols has led to better follicular activity (Heidari et al., 2017), increase in the uterine tonicity and phagocytic capacity of PMNCs via leukotriene B4 (LTB4) production under the influence of PGF2α administration (Lewis, 2004; Sahadev et al., 2019). The findings were in agreement with Heidari et al. (2017) who reported higher ovulatory response and conception in cows having follicle diameter greater than 15 mm following treatment with MG6G and MG6GP estrus synchronization protocols as compared to control group cows. Other researchers have also reported better follicular response i.e. initiation of follicular activity post-partum along with higher follicular diameters, after application of presynchronization protocols (Coto, 2016; Carvalho et al., 2014; Dirandeh et al., 2018). However, the pre-ovulatory follicle size remained affected by the presence of SCE which was not similar to the findings of Coto (2016).

Post-partum sub-clinical endometritis affects the fertility of cows negatively, thus, leads to poor reproductive performance (Carneiro et al., 2014) and in the process, deteriorates the economy of dairy farmers and entrepreneurs. The current study was in concurrence with findings of various researchers (Plontzke et al., 2010; Barrio et al., 2015; Rinaudo et al., 2017; Sharma et al., 2018) who reported higher number of days to first AI (77-93 vs 68-85 days) and subsequently, conception (154-166 vs 113-119 days) probably due to impaired sperm transport and storage, ovulation and zygote development (Gilbert, 2011). Similarly, number of inseminations per conception was higher in SCEP cows as compared to SCEN cows although the difference was not statistically significant ($p>0.05$) and were quite similar to the findings of Barrio et al., (2015). On the contrary, Carneiro et al., (2014) and Coto and Lucy (2018) reported no effect of SCE on conception rate although pregnancy losses were greater by day 32 after insemination.

CONCLUSION

The present study concluded that the treatment with modified estrus synchronization protocols proved to be effective in decreasing the uterine inflammation at uterine perfusion, PMNCs proportion and serum inflammatory markers. Also, the development of good follicles after estrus synchronization attenuated the deleterious effect of sub-clinical endometritis on post-partum reproductive performance of dairy cows reared in the ever-growing dairy industry.

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AUTHOR CONTRIBUTION STATEMENT

All the co-authors have contributed in designing methodolgy, supervision of project from time to time as well as writing and reviewing the original draft of research paper. However, no special funding was received for the project.

COMPETING INTEREST STATEMENT

It is verified again that authors have no conflict of interest to declare.

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