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B-Mode and Color Doppler Sonographic Appearance of Pelvic Tendon, Ligament and Uterine Blood Flow in Non-pregnant and Heavy Pregnant Dairy Cows

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Abstract:
This study was carried out to provide comparative B-mode and Doppler ultrasonographic description of pelvic tendon, ligaments, middle uterine artery and placentomes in non-pregnant and heavy pregnant cows. It also, screens pregnancy associated changes like hemodynamics of middle uterine artery and serum concentrations of estrogen and progesterone. Forty pluiparous dairy cows of native breeds were scanned. The animals were separated into two groups of 20 cows, first group was non-pregnant and second group was heavy pregnant at 9th month of gestation. The examination was performed using multiple imaging B-mode and color Doppler ultrasonography. Pelvic tendon and ligaments including dorsal branch of the dorsal sacroiliac ligament- thoracolumbar fascia combination (D-DSIL-TLF), lateral and ventral branches of the dorsal sacroiliac ligament (L-DSIL and V-DSIL respectively), and sacrosciatic ligament (SSL) as well as the middle uterine artery (MUA) and placentomes were monitored. Serum estrogen and progesterone concentrations were evaluated. Results revealed that pregnancy greatly influenced doppler indices and diameter of MUA, serum estrogen and progesterone concentrations, and measurements of pelvic ligaments except the thickness and cross -sectional area of D-DSIL- TLF combination. The obtained results could be used as a guide for future studies dealing with monitoring normal and abnormal pregnancy in cows.

INTRODUCTION

Dystocia and its magnitudes are common problems in bovine breeding. It is implicated in high calf mortality with negative impacts on subsequent pregnancies. It is also associated with greater cow culling rate, lower fertility, reduced milk production, and high expenses of veterinary care services (Fourichon *et al.,* 2000; Oliveira and Gheller, 2009). There is a direct relation between the ease of delivery and body conformation, pelvic shape, dimensions and hormonal control of the dam in relation to calf birth weight besides its position and presentation (Naazie *et al.,* 1989; Nogalski, 2002; Mee *et al.,* 2011). Delivery occurs via interconnection between endocrine, neuronal, and mechanical pathways (Knobiland Neill, 1994; Hafez and Hafez, 2000). Released hormones principally estrogen and progesterone were moderated during bovine pregnancy and were convoluted in expulsion of alive fetus (Kindahl *et al.,* 2002).

Doppler ultrasonography is valued equipment in veterinary Obstetrics. It was used in monitoring of uterine blood supply in heifers (Heppelmann *et al.,* 2013), divergence between uterine torsion and normal pregnancy in buffaloes (Devender *et al.,* 2018), segregating between pyometra and normal pregnancy bitches (Pati *et al.,* 2019), discrepancy of compromised pregnancy in cows (Hassan *et al.,* 2020), and evaluating the changes of uterine and ovarian perfusion during puerperium (El-sherbiny *et al.,* 2020). Throughout pregnancy, structural and functional changes normally occur in the uterine vascular supply based on state of fetal nutritional needs. It involves hemodynamic changes to ascertain ideal blood supply to the growing placenta/fetus (Ferrel, 1991).

Assessment of internal pelvic measurements and pelvic structures particularly bones and joints was gained a lot of concern during the past decades (Nogalski, 2002; Bennett *et al.,* 2008; Oliveira and Gheller, 2009; Sharshar *et al.,* 2020). To our knowledge, little attention was paid to pelvic ligaments and tendons despite of their significance for normal parturition. Additionally, there is a few data regarding indices of blood flow and changes in middle uterine artery particularly during late period of gestation in dairy cows. Therefore, the current study aimed to monitor the ultrasonographic B-mode description of pelvic ligaments, tendon, middle uterine artery and placentomes. Besides, it aimed to provide a Doppler sonographic scanning of uterine blood flow in non-pregnant and heavy pregnant dairy cows.

MATERIALS AND METHODS

Ethical statement

The present study was approved by institutional Animal Care and Use Committee of Faculty of Veterinary Medicine, University of Sadat City, Egypt (protocol no:VUSC-030-1-20).

Animals and study design

This study was carried out on a total number of 40 healthy pluriparous dairy cows of native breed (*Baladi breed*) reared on the educational farm of the Faculty of Veterinary Medicine, University of Sadat City, Egypt, from November to December 2020. The mean of animals age was 7 years (range from 5 to 9 years) and the mean of body weight was 400 kg (range from 375 to 450 kg)and the body condition score ranged from (3.5 to 4) on a scale of 5. Cows were separated to two groups each of 20 cows according to the reproductive state (1st group was non-pregnant and the 2ndgroup was pregnant at 9th month). Following breeding soundness examination, animals were apparently normal, vaccinated dewormed and free from reproductive disorders. Animals were kept under same management conditions and were housed in free stall barn and were fed a well-balanced ration with free access to water.

Examinations

B-mode ultrasonography of pelvic ligaments and tendon

Ultrasonographic system (EsaoteMyLab™One VET, Italy) equipped with 6.6 to 18 MHz micro-convex, linear array tendon and rectal linear probes were used for scanning. Animals were secured in a stanchion, hairs overlaying the pelvis were clipped, cleaned and the skin was covered by gel. Evacuation of rectum from feces was done before trans-rectal scanning.

Pelvic ligaments involving dorsal branch of the dorsal sacroiliac ligament (D-DSIL), lateral branch of the dorsal sacroiliac ligament (L-DSIL) and Sacrosciatic ligament (SSL), as well as pelvic tendon including thoracolumbar fascia(TLF) were scanned using B-mode ultrasonography. Images were gained by longitudinal and transverse sections for each structure. Probe type and placement, adopted frequency, depth and assessed variables for each structure were summarized in Table 1.

The imaging procedures were conducted following basics previously described by (Head, 2014; Whitcomb and Vaughan, 2015; Sharshar *et al.,* 2020).

Table 1. Probe type, placement adopted frequency, depth and assessed variables for each structures.

Examined structures	Probe type	Frequency	Depth	Placement of the probe	Assessed variables
Dorsal branch of the dorsal sacroiliac ligament-thoracolumbar fascia combination (D-DSIL-TLF)	Linear tendon	10 MHz	4cm	At the proximal part of sacral tuber	Shape, echogenicity, cross sectional area and thickness
Lateral branch of the dorsal sacroiliac ligament (L-DSIL)	Linear tendon or Micro-convex	8 MHz	10 cm	The probe was moved laterally in a caudo-ventral direction starting at the caudal portion of the sacral tuber	Shape, echogenicity, and thickness.
Sacrosciatic ligament (SSL)	linear tendon or Micro-convex	10 MHz	7 cm	The probe was placed at its origin at the lateral aspect of the sacral transverse processes and directed caudo-ventrally to its insertion at ischial tuber	Shape, echogenicity, and thickness
Ventral branch of the dorsal sacroiliac ligament (V-DSIL)	Linear tendon or Micro-convex	10 MHz	7 cm	Probe was placed transrectally	Shape, echogenicity, and thickness.

B-mode and Doppler ultrasonography of middle uterine artery and placentomes

The scanning was conducted on the same basis as previously established by (Varughese *et al.,* 2013; El-sherbiny *et al.,* 2020; Hassan *et al.,* 2020). A rectal spectral-wave Doppler ultrasound equipped with 10 MHz linear-array transrectal transducer (EsaoteMyLab™One VET, Italy) was used. The cross-sectional dimensions of the middle uterine artery and placentomes (mm) were measured. The blood flow parameters, pulsatility index (PI) and resistance index (RI) were determined using automatic options on the device and the images were stored.

Determination of estrogen and progesterone serum concentrations

Blood (5 ml) was collected from the jugular vein of animals. Samples were kept in a slope direction in the refrigerator for 12 h, clotted samples were centrifuged for 15 minutes at 3000 rpm and collected serum was stored at -20 °C in Eppendorf tubes until analysis. Serum level of estrogen and progesterone was detected in serum samples using ELISA kits (Calbiotech, Austin, Springer valley, CA, 91978, USA) following the micro-well method and the OD (optical density) absorbance has been adjusted at 450 ±10 nm.

Statistical analysis

Statistical analysis was performed with GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA). The measured variables were compared among pregnant and non-pregnant animals using Independent Samples *t*-Test. Data are presented as mean± standard error (SE). The level of significance was set at $P<0.05$.

RESULTS

The dorsal branch of the dorsal sacroiliac ligament-thoracolumbar fascia combination appeared during transverse scan as curvilinear echogenic structure divided into two parts the lateral part representing the dorsal branch of the dorsal sacroiliac ligament which appears slightly echogenic convex structure covering the hyperechoic sacral tuber. The medial branch appears as V-shape echogenic structure extended medially to the proximal aspect of the dorsal spinous process of the first sacral vertebrae (Figure 1A). In longitudinal scanning, both structures appeared as echogenic layer extended cranio-caudally covering sacral tuber. It divided into two echogenic layers at the cranial edge of the sacral tuber while proceeding caudally as a single cord like structure (Figure 1B). The thickness of D-DSIL- TLF was 3.65±0.03 mm in pregnant cows and 3.69±0.02 mm in non-pregnant ones without significant difference between them (Figure 2A).

The mean cross-sectional area of both structures in pregnant cows was 1.36±0.02 cm^2 while in non-pregnant cows was 1.4±0.01 cm^2 with no significant difference between them (Figure 2B). The L-DSIL appeared in both pregnant and non-pregnant cows as linear echogenic structure extended caudo-laterally in a slightly ventral direction to insert at the lateral aspect of the sacral crest which appears as hyperechoic arch (Figure 1C). Its thickness was significantly lower in pregnant (3.24±0.37 mm) compared to non-pregnant (5.36±0.33 mm) ones (Figure 2C). During longitudinal scan of SSL, appeared in both pregnant and non-pregnant cows as echogenic flat sheet interspersed with an-echoic areas. It is originating from a hyperechoic linear structure representing the lateral aspect of the sacral transverse process (Figure 1D) and extending caudo-ventrally to insert at the Ischiatic tuber which appears as hyper echoic curvilinear structure (figure 3A). The thickness of SSL was significantly lower in pregnant (6.32±0.2 mm) compared to non-pregnant (8.14±0.29 mm) cows (Figure 2D). In longitudinal scan, the V-DSIL appeared as hyperechoic sheet joining between two smooth hyperechoic arches representing the iliac wing and the first sacral transverse process and pounded the sacroiliac joint ventrally (Figure 3B). Its thickness was also significantly lower in pregnant (2.7±0.31 mm) than non-pregnant (3.77±0.07 mm) cows (Figure 4A).

Characterization and hemodynamic changes of uterine blood flow

Regarding uterine blood flow, during rectal palpation by hand there was a characteristic change in the pulse wave of middle uterine artery (fremitus) in heavy pregnant than non-pregnant dairy cows. Scanning B-mode and Doppler sonography of blood flow of middle uterine artery of non-pregnant dairy cows revealed its small diameter (0.82±0.02 cm) and the existing low systolic velocity (Figure 3C, D & 4B). On the other hand, there was a significant increase in the diameter (1.54±0.1 cm) of MUA with peak systolic velocity waveform of its blood flow with higher blood perfusion (represented by the bright orange pixels on the center of the artery) in heavy pregnant cows (Figure 5A, B & 4B). The Doppler indices (PI and RI), were significantly lower in heavy pregnant (1.5±0.04 and 0.63 ± 0.03 respectively) compared to non-pregnant (2.26±0.06 and 0.91±0.01 respectively) cows.

Characterization and hemodynamic changes of placentom

The placentom of the gravid horn showed a moderate blood perfusion as evidenced by bright orange pixels on the center of the placentom (Figure 5C&D). The blood flow pattern of placentom exhibited both the systolic and diastolic peaks using color doppler.

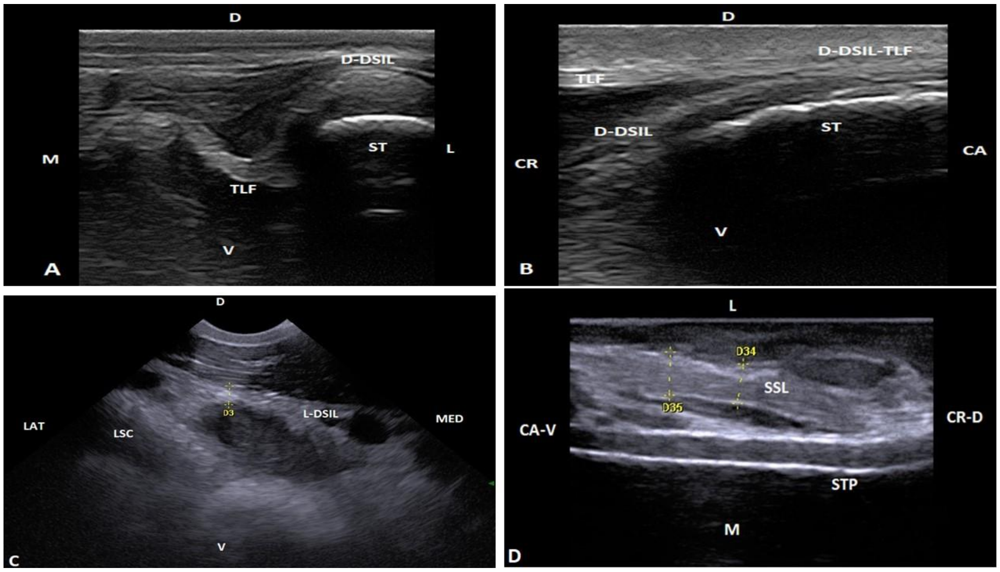

Fig. 1. (A): Transverse sonogram of the dorsal sacroiliac region (linear tendon probe, 10 MHz and 4cm depth) of 7 years old pregnant cow. The dorsal branch of the dorsal sacroiliac ligament (D-DSIL), sacral tuber (TS), thoracolumbar fascia (TLF), D: dorsal direction. V: ventral direction. M: medial. L: lateral.

(B): Longitudinal sonogram at the dorsal aspect of sacral tuber (ST) of the same cow in figure 1A showing; the dorsal aspect of the dorsal sacroiliac ligament (D-DSIL), thoracolumbar fascia (TLF). D: dorsal direction. V: ventral direction. CR: cranial direction. CA: caudal direction

(C): Longitudinal sonogram of the lateral branch of the dorsal sacroiliac ligament (L-DSIL) (8 MHz micro-convex probe, 10 cm depth) of 7 years old non-pregnant cow (LSC) lateral sacral crest. LAT: lateral direction. MED: medial direction. D: dorsal direction. V: ventral direction.

(D): Longitudinal sonogram of the sacro-sciatic ligament(SSL) at its origin (10 MHz linear tendon probe, 7 cm depth) of 9 years old non-pregnant cow. (STP) sacral transverse process. (L) lateral, (M) medial, (CA-V) caudo-ventral, (CR-D) cranio-dorsal.

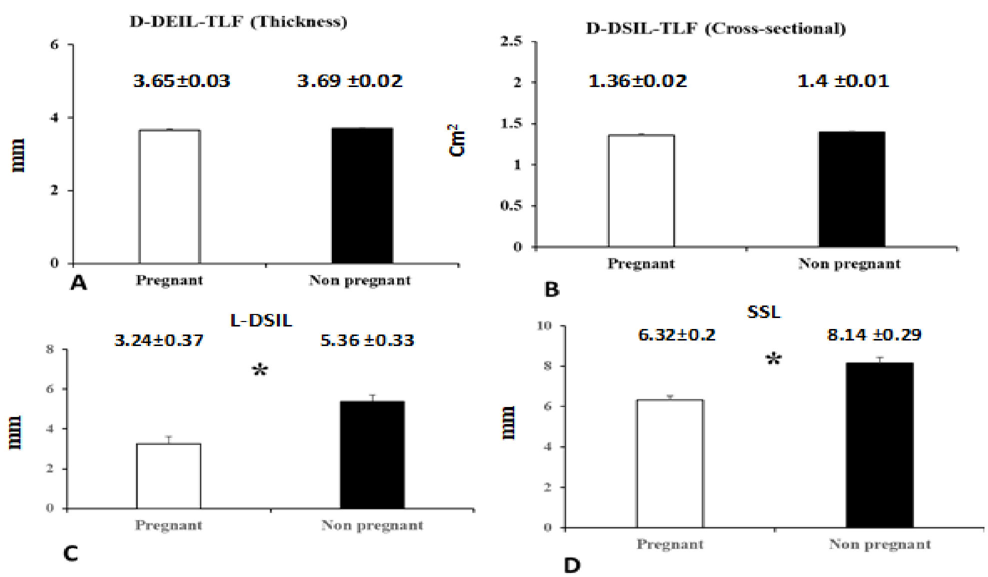

Fig. 2. (A & B): Mean thickness and cross-sectional area of dorsal branch of the dorsal sacroiliac ligament- thoracolumbar fascia combination (D-DSIL-TLF) in pregnant and non-pregnant cows; (C): Thickness of the lateral branch of the dorsal sacroiliac ligament (L-DSIL) in pregnant and non-pregnant cows; (D): mean thickness of the sacrosciatic ligament (SSL) in pregnant and non-pregnant cows. All data are presented as mean ±SE.*Values are significantly different between pregnant and non-pregnant cows.

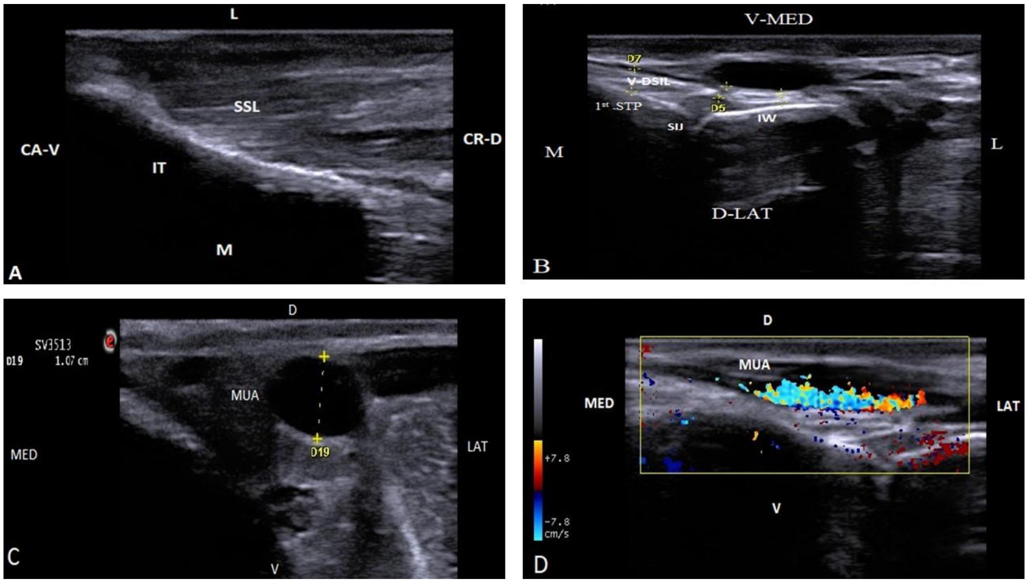

Fig. 3. (A): Longitudinal sonogram of the sacro-ischiatic ligament at its insertion (SSL) of the same animal in Figure 1D. (IT) ischial tuber. (L) lateral, (M) medial, (CA-V) caudo-ventral, (CR-D) cranio-dorsal; (B): Longitudinal sonogram of the ventral branch of the dorsal sacroiliac ligament (V-DSIL) of 5 years old non -pregnant cow. (IW) iliac wing, (1st STP) sacral transverse process of the first sacral process, (SIJ) sacroiliac joint, (V-MED) ventro-medial, (D-LAT) dorsolateral; (C&D): Ultrasonographic monitoring of middle uterine artery of non-pregnant cows. (C): B-mode ultrasonography; (D): Doppler sonography.

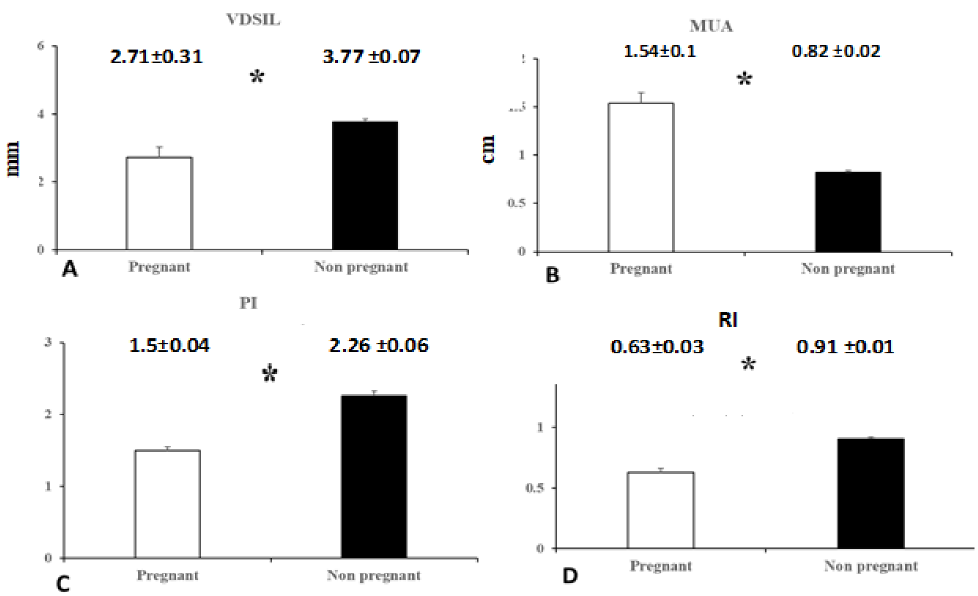

Fig. 4. (A): mean thickness of the ventral branch of the dorsal sacroiliac ligament (V-DSIL) in heavy pregnant and non-pregnant cows; (B): mean diameter of the middle uterine artery (MUA) in heavy pregnant and non-pregnant cows; (C &D): Pulsatility index (PI) and resistance index (RI) in heavy pregnant and non-pregnant dairy cows. Data are presented as mean± SE. *Values are significantly different between pregnant and non-pregnant cows.

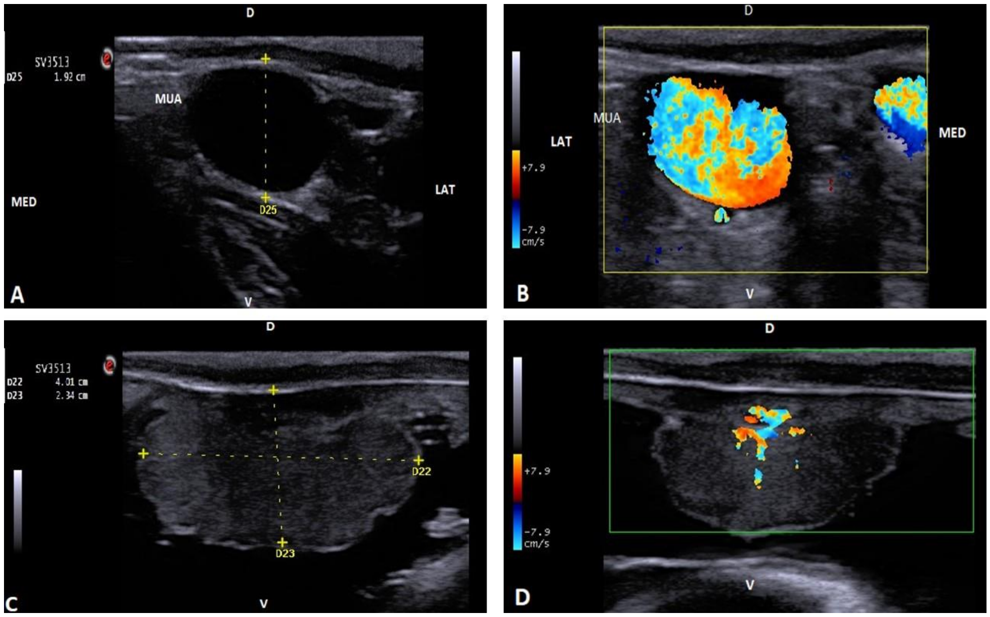

Fig. 5. (A &B):Ultrasonographic monitoring of middle uterine artery of heavy pregnant cows(10 MHz linear-array transrectal transducer 7 cm depth). (A): B-mode ultrasonography; (B): Doppler sonography. (C&D): Ultrasonographic monitoring placentom of heavy pregnant dairy cows (10 MHz linear array transrectal transducer 7 cm depth). (C): B-mode ultrasonography; (D) Doppler sonography.

Hormonal concentration

Serum estrogen concentration was significantly higher in heavy pregnant (170 ± 3.5 Pg/ml) cows compared to non-pregnant (7.5 ± 0.67 Pg/ml) ones without. Serum progesterone concentration showed slight increase in heavy pregnant cows than non-pregnant ones with no significance difference (5.14 ± 0.67 and 3.35 ± 0.89 ng/ml) between them (Figure 6 A&B).

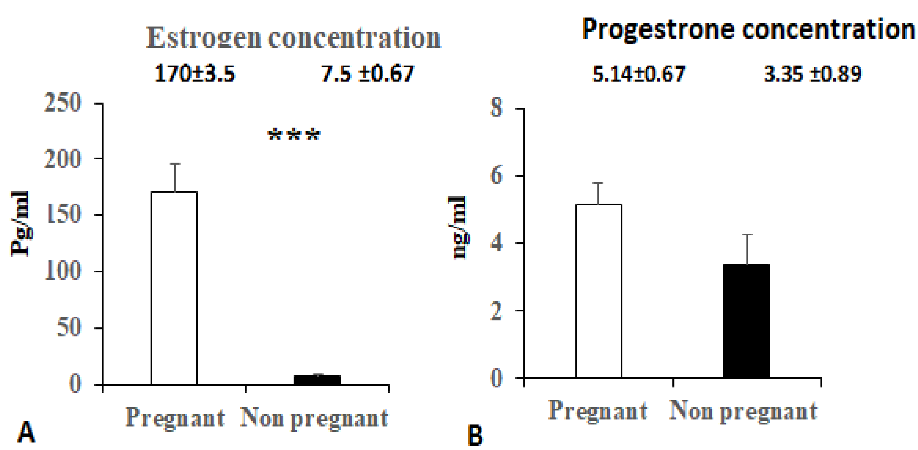

Fig. 6. Serum estrogen (Pg/ml) and progesterone (ng/ml) concentrations of heavy pregnant and non-pregnant dairy cows. Data are presented as mean±SE. *Values are significantly different between pregnant and non-pregnant cows.

DISCUSSION

Ruminant pelvis is a multiple functional osteoligamentous structure (Oliveira *et al.,* 2003; Silva *et al.,* 2019). It is an osseous ring encircles the birth canal and limits its expansion (Tyczka, 1998). As a result of continuous breeding with the general direction to increase body and udder masses, pelvic structure is frequently subjected to numerous undesirable changes (Jalakas *et al.,* 2000; Bennett *et al.,* 2008). Consequently, bovine pelvis would become gradually inappropriate for normal delivery (Duce *et al.,* 2002; Nahkur *et al.,* 2003). Throughout reproductive cycle, there is an increasing load upon the supporting tissues of the reproductive organs due to the simultaneous changes in their weight and size (Oliveira and Gheller, 2009; Majida *et al.,* 2010; Hopper, 2014; Herring, 2014). Pelvic structure and diameters of cows was gained a lot of concern during the past decades to expect those at higher risk of dystocia (Laster, 1974; Johnson *et al.,* 1988).

In adult cow, the hip bones are joined osseously and the pelvic symphysis is ventrally reinforced by the symphysial crest. Therefore, the pelvic cavity can be widened at the time of delivery by relaxation of the broad sacro-ischiatic ligament and the sacroiliac joint (Jalakas and Saks, 2001). Our results revealed that the thickness of the lateral and the ventral branches of the dorsal sacroiliac ligament and the SSL were significantly lower in heavy pregnant cows than non-pregnant ones. This might be attributed to alteration of ligaments structure as a result of increasing the level of the circulating steroidal hormones particularly estrogen and progesterone at the last trimester of pregnancy (Henricks *et al.,* 1972; Derivaux and Ectors, 1984; Kindahl *et al.,* 2002). According to authors' opinion, reduction of pelvic ligaments thickness could be also an indicative for its possible relaxation because of proximity of delivery with subsequent widening of the sacroiliac joint and increments of pelvic width at its inlet and outlet respectively, supporting of our hypothesis (Jalakas and Saks, 2001).

To our knowledge this might be the first report to provide complete ultrasonographic description of pelvic ligaments and tendon in non-pregnant and heavy pregnant cows. Only Sharshar et al. (2020) described some of them, but not all including D-DSIL- TLF combination and L-DSIL. According to this study, the D-DSIL- TLF combination and L-DSIL appeared as moderately echogenic curvilinear or linear structure, respectively. This data were found in accordance with previously published reports in equine (Engeli *et al.,* 2006; Sharshar *et al.,* 2018) and cattle (Sharshar *et al.,* 2020). The SSL appeared at its origin as moderately echogenic sheet interspersed with small anechoic areas. This might be attributed to its anatomical configuration in which the ligament attached to more than one structure at its origin occluding the last sacral and coccygeal vertebrae (Nahkur *et al.,* 2003). In the current work, the thickness of both D-DSIL-TLF and L-DSIL was estimated in heavy pregnant and non-pregnant cows. Our results found in accordance with previously published data (Sharshar *et al.,* 2020).

Doppler trans-rectal ultrasonography was established as non-invasive tool to assess the uterine blood dynamics throughout pregnancy in buffaloes as well as to monitor hemodynamic changes in pregnancy disorders (e.g. abnormalities in uterine blood flow/placenta/fetus) (Varughese *et al.,* 2013). In the present study, Doppler ultrasound was efficient in determining and comparing the uterine blood flow in heavy pregnant and non-pregnant cows. Scanning B-mode and Doppler ultrasonography of blood flow of middle uterine artery of non-pregnant dairy cows revealed small diameter of MUA and low systolic velocity. These findings agreed with previous study in cows (El-sherbiny *et al.,* 2020) where diameters of uterine and ovarian arteries declined gradually from the 1st week until the end of 6th week after parturition. Additionally, the uterine perfusion was reduced significantly at the 1st week postpartum in dairy cows (Krueger *et al.,* 2009; Heppelmann *et al.,* 2013).

Here, we reported a significant progress in the diameter of middle uterine artery and peak systolic velocity waveform of its blood flow in heavy pregnant cows. These findings were in harmony with Hassan et al. (2020) who reported an increment in the diameter of uterine artery and blood flow as pregnancy advances. Based on both studies along with the decrease of blood flow in the middle uterine artery in case of uterine torsion in cattle (Singh *et al.,* 2016; Devender *et al.,* 2018), it could be elucidated that increased blood flow of middle uterine artery is a characteristic Doppler finding for pregnancy**.**

The placentomes of the gravid horn showed moderate blood perfusion represented by the bright orange pixels on the center of the placentom. These results were parallel to that previously recorded by Hassan et al. (2020). Therefore, fetal and maternal environment were indispensable factors for the maintenance of successful pregnancy (Spencer *et al.,* 2006).

In the present work, there was a significant difference between heavy pregnant and non-pregnant dairy cows in the measured Doppler indices (PI and RI).Similar results were obtained by (Varughese *et al.,* 2013; Singh *et al.,* 2017; Hassan *et al.,* 2020). The obtained PI and RI values were significantly lower in heavy pregnant compared to non-pregnant cows. These findings could be indicative for lower resistance to blood flow as well as better uterine perfusion as a result of pregnancy (Silva, 2011). In agreement with our findings, in the study of Bollwein et al. (2002), the PI and RI parameters were decreased continuously during the first 8-month of gestation. On the other hand, (Panarace *et al.,* 2006) showed significant changes in PI and RI were not detected after 26th week of gestation in cows. This discrepancy might be attributed to fetal, cellular, hormonal, nutritional and molecular changes that predispose to different hemodynamic patterns during late pregnancy in cows, which need future studies.

CONCLUSION

The present study elucidated the clinical applicability of ultrasound in determining pregnancy associated changes in pelvic ligamentous and tendinous structures. It could be also a valuable tool to ascertain baseline data of uterine hemodynamic changes (PI and RI) and diameter of MUA in late pregnancy in correlation with non-pregnant dairy cows. Moreover, it provided information about the hormonal difference between non-pregnant and heavy pregnant cows. These findings could be useful for dam and fetus before further obstetrical handling that could be evaluated by trans-rectal color Doppler ultrasonography. Further study is needed to evaluate such B-mode and Doppler data in cows delivered normally and those with dystocia.

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CONFLICT OF INTEREST

Authors declare have no conflicts of interest.

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