RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume 41 Issue 1: 1-7 (March 2022)

Color Doppler Ultrasonographic Characteristics of Embryonic Development and Mortality in Dairy Cows

Mohit Mahajan, Madhumeet Singh, Akshay Sharma, Pravesh Kumar

10.18805/ajdfr.DR-1670

ABSTRACT

Background: Embryonic mortality is one of the major factors leading to reproductive failure, decreased fertility and delayed pregnancy in cattle.

Methods: The study was aimed for investigating the incidence of embryonic mortality(EM), differentiate embryonic development and mortality based on color Doppler ultrasonographic characteristics. Different characteristics correlated were corpus luteum(CL) vascularity, embryonic development and EM features, monitored on day 21 post-insemination.

Result: The overall incidence of EM was recorded to be 25%, with 15% and 10% mortality occurred between days 21-28 and 28-35 of gestation. Color Doppler analysis stated that, the CL in pregnant cows had a higher percentage of luteal tissue with positive Doppler signals on initial examination (day 21) differing significantly ($P<0.01$) from non-pregnant and consistently increased till the end of the examination. However, cows with EM had a significantly lower CL vascularity than pregnant ($P<0.05$) but significantly higher ($P<0.05$) than non-pregnant cows on day 21. Embryos from pregnant cows (detected by 28 days) increased in size along the days of examination. However, cows diagnosed with EM showed signs (day 28), which included reduced intra-uterine allantoic fluid, absence of embryo proper with an embryonic heartbeat, hyperechoic free-floating embryonic debris in the uterus and distorted allanto-chorionic membrane. In conclusion, the present study firmly supports color Doppler ultrasonography in diagnosis of pregnant cows based on CL vascularity, visualizing the different characteristics signs of EM, and detecting non-pregnant animals undergoing EM as early as day 21.

Key words: Color doppler ultrasonography, Corpus luteum vascularity, Embryonic mortality, Embryonic development, Jersey cows.

INTRODUCTION

Embryonic mortality in the reproductively sound animals, is one of the major factors of reproductive failure, decreased fertility, delayed pregnancy in cattle, fewer calves born, reduced milk production and slower genetic progress, especially, in repeat breeder females with late embryonic mortality (Diskin *et al*. 2016). Generally, fertilization rate with artificial insemination (AI) ranges from 85-90 per cent (Sreenan *et al*. 2001; Sharma *et al*. 2019), whereas, conception rate in dairy herds is about 52 per cent (Bansal *et al*. 2019) and declines an additional 10 per cent in lactating dairy cows (Whitlock and Maxwell 2008). Embryos are most susceptible to mortality during their development from morula to blastocyst stage *i.e*. the prehatching period (Shirasuna *et al*. 2012).

Color Doppler is a relatively new technique employed during recent times in cattle reproduction (Sharma *et al*. 2019a). Ultrasonography has been the best non-invasive method to study the characteristics of embryonic viability and mortality from day 25 post-AI (Sharma *et al*. 2018b) and also, the corpus luteum (CL) morphology (Ginther 2014) and functionality (Jokubkiene *et al*. 2006). Therefore, the present study envisaged the embryonic viability/mortality and luteal blood flow characteristics in dairy cows.

MATERIALS AND METHODS

The study was conducted on twenty-nine (n=29) cross bred Jersey cows from March to December 2019 at Instructional Livestock Farm Complex (ILFC), CSK Himachal Pradesh Agricultural University, Palampur. Animals were housed in concrete dry sheds and fed with both green fodder and dry fodder/hay. Recommended dose of concentrate and mineral mixtures were supplemented in fodder/hay and clean drinking water was available free choice. All the animals had sound health with no apparent clinical signs of any disease or deficiency. Trans-rectal ultrasonography (Mindray Z5 VET) of the genital tract and ovaries was done with a linear rectal probe at 7.5 MHz frequency from day 21 to 42 of gestation at weekly intervals in brightness mode (B-mode) and color

Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062, Himachal Pradesh, India.

Corresponding Author: Mohit Mahajan, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062, Himachal Pradesh, India. Email: mohitmahajan108@gmail.com

How to cite this article: Mahajan, M., Singh, M., Sharma, A. and Kumar, P. (2022). Color Doppler Ultrasonographic Characteristics of Embryonic Development and Mortality in Dairy Cows. Asian Journal of Dairy and Food Research. 41(1): 1-7. DOI: 10.18805/ajdfr.DR-1670.

Submitted: 05-04-2021 **Accepted:** 14-06-2021 **Online:** 28-07-2021

Doppler mode. Animals that were diagnosed as non-pregnant were scanned up to day 28 post insemination. Various characteristic echographic structures on ovaries and embryonic characteristics were visualized, recorded and analysed.

Corpus luteum (CL) was recorded on an ovarian map and its measurements were performed using ultrasound machine (internal digital callipers) for each examination. Blood perfusion to the corpus luteum was measured; cross-sectional images of the maximal area of the CL (square millimetre) as vascular transverse section were recorded. The mean CL vascularity was calculated as an average of three cross-sectional images using the grid technique (Luttgenau and Bollwein 2014).

The diagnosis of embryonic mortality was based on findings of Romano (2004) and Gabor *et al*. (2016). Numeric data for all the parameters are expressed as mean±SD and statistical analysis was carried out using one-way ANOVA (Statistical Analysis Software® 9.2 TS Level version 2M2 for windows).

RESULTS AND DISCUSSION

In the present study, the overall incidence of embryonic mortality was recorded to be 25% with 15 and 10% mortality occurred between days 21-28 and 28-35 of gestation, respectively (Table 1). In accordance with our findings, Gatea *et al*. (2018) reported 23% embryonic mortality between days 30-60 of gestation, whereas, other researchers reported a lower incidence of 13% between days 32-45 (Fricke *et al*. 2016), 18.6-19.75% between days 24-42 (Pohler *et al*. 2016) and 17.0% between days 28-32 of gestation (Hernandez *et al*. 2012) in dairy cows. However, a higher incidence of 40-56% and 42.7% embryonic mortality in moderate and high yielding cows was reported between day 0-42 of gestation by Diskin and Morris (2008) and Pereira *et al*. (2013), respectively.

The size of CL was maximum at the beginning of examination (day 21), remained nearly constant over the period of examination in pregnant and embryonic mortality cows, but differed significantly (P<0.01) from non-pregnant cows (Table 2). In accordance with our findings, Luttgenau and Bollwein (2014) also reported a reduction in the CL size in cows that supervenes embryonic mortality during early gestation. Contrarily, Herzog *et al*. (2011) concluded that size of CL did not alter much after day 18 in cows with embryonic mortality *i.e*. after maternal recognition of pregnancy. The decrease in CL size is indicative of the loss of luteal parenchymal cells (Tamura *et al*. 2008) leading to an overall reduction in luteal tissue in subsequent examinations. Ultrasonographic studies of the corpus luteum revealed that it displays a dynamic pattern of development and regression based on the physiological state of the genitalia/state of pregnancy or non-pregnancy and embryonic mortality (Luttgenau and Bollwein 2014). Scully *et al*. (2014) observed a significant difference between CL size of pregnant and non-pregnant cows and considered it as a possible marker for a functional CL. However, some researchers do not consider the size of the CL as a reliable indicator of pregnancy and its functional status (Scully *et al*. 2014).

The vascularity of CL was initially along the outer border, in a ring-like pattern, which tends to increase towards the centre along with the increase in gestation length (Fig 1a, 2a, 3a, 4a). The vascularity in pregnant cows showed a higher percentage of luteal tissue with positive Doppler signals on initial examination (Day 21) differed significantly (P<0.01; P<0.05) from non-pregnant and embryonic mortality cows and continued to increase till the end of examination (Table 2). Also, the luteal tissue vascularity decreased constantly from day 28 until the end of examination in cows diagnosed with embryonic mortality (Fig 1b, 2b, 3b, 4b). In agreement with our findings, Scully *et al*. (2014) reported that pregnant cows have significantly higher vascularity as compared to non-pregnant cows on all days of examination. Similarly, Pinaffi *et al*. (2017) reported that vascularity of CL in pregnant cows tends to increase along gestation. The high vascularity demarcated the active functional state of CL for progesterone production (Luttgenau and Bollwein 2014). The CL blood flow is associated with luteal regression at end of the estrous cycle between day 16-20 post-ovulation (Pinaffi *et al*. 2017). Thus, the diagnosis of state of pregnancy as early as day 21 of gestation could be done on the basis of CL vascularity being present or absent (Pancarci *et al*. 2012).

The uterine horn lumen in pregnant cows increased throughout the period of examination (Table 2), along with the developing embryo proper with clear uterine fluid within the lumen (Fig 3a, 4a, 5a, 6a). The ultrasonographic appearance of uterine horns with uterine fluid was due to the activity of secretory glands and microvasculature associated with endometrial development and implantation of the embryo (Gray *et al*. 2001). The embryonic size had large variations among individual animals (Pohler *et al*. 2014). The embryo in pregnant cows was detected as early as 28 days of gestation (Fig 6a), with the size of the embryo increasing gradually in pregnant cows over the period of

Table 1: Incidence of embryonic mortality in dairy cows.

No. of total pregnant cows	Day at mortality							
	Day 21-28		Day 28-35		Day 35-42		Overall	
n=20	No. of cows	% mortality	No. of cows	% mortality	No. of cows	% mortality	No. of cows	% mortality
	3	15.00	2	10.00	0	00.00	5	25.00

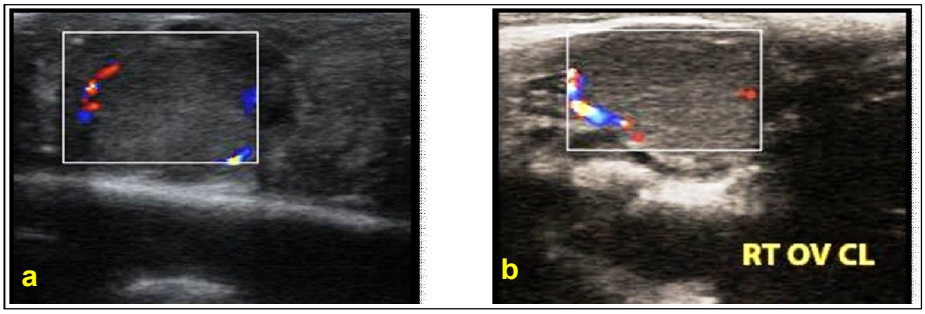

Fig 1: Color Doppler image of CL in pregnant cows and cows with embryonic mortality on day 21;
[a] Pregnant - color Doppler signals developing along the border of CL, [b] Embryonic mortality - weak color Doppler signals.

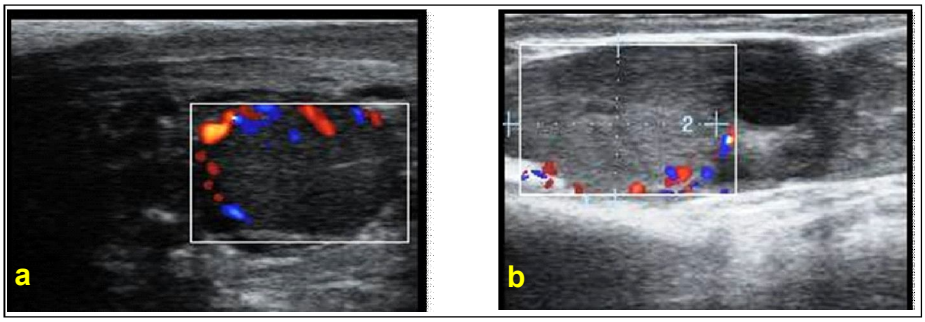

Fig 2: Color Doppler image of CL in pregnant and cows with embryonic mortality on Day 28;
[a] Pregnant - increase in Doppler signals around the borders of corpus luteum, [b] Embryonic mortality - weak color Doppler signals.

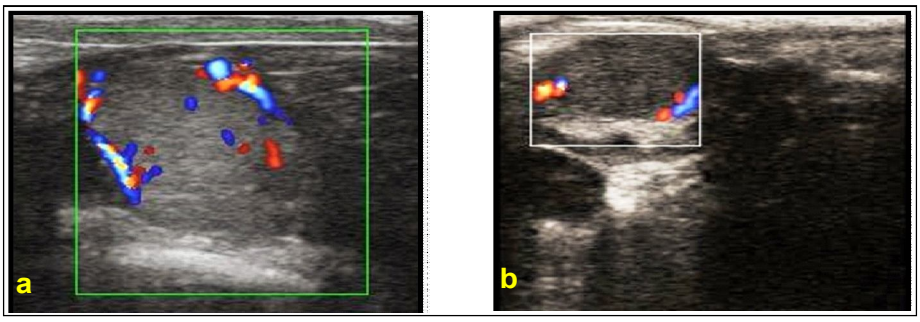

Fig 3: Color Doppler image of CL in pregnant and cows with embryonic mortality on Day 35;
[a] Pregnant - positive color Doppler signals along the periphery with more signals towards the centre of CL,
[b] Embryonic mortality- reduction in CL size and color Doppler signals.

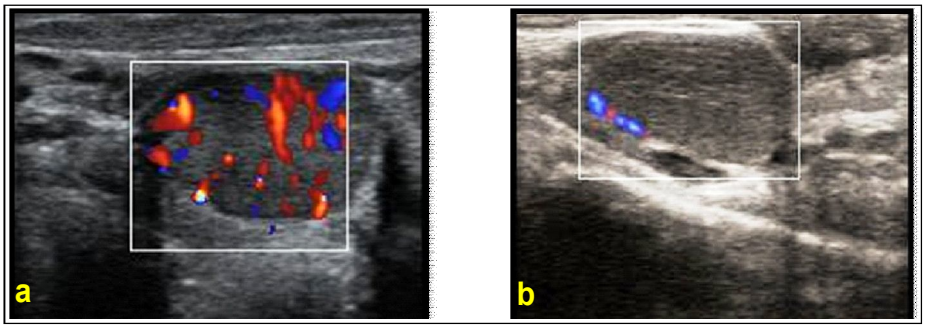

Fig 4: Color Doppler image of CL in pregnant and cows with embryonic mortality on Day 42;
[a] Pregnant - strong color Doppler signals in the periphery and centre of the CL, [b] Embryonic mortality - absence of Doppler signals.

examination (Fig 7a, 8a). The amniotic sac, which surrounds the embryo, increased concurrently with the size of the embryo in pregnant cows (Table 2). Therefore, diagnosis of pregnancy based on ultrasonographic visualization of the embryo was possible only 28 days after successful AI, as the embryo lies closely adhered to the uterine wall which is concurrent with our findings (Gnemmi 2004).

The cows diagnosed with embryonic mortality showed distinct ultrasonographic signs from day 28 of gestation, which included reduced intra-uterine allantoic fluid and absence of embryo proper with an embryonic heartbeat (Fig 8b) in contrast to the findings recorded in pregnant cows (Fig 8a). Uterine lumen was filled with hyperechoic free-floating structures representing embryonic debris (Fig 6b, 7b, 8b), embryonic remnants with ill-defined embryonic outline and distorted allanto-chorionic membrane (Fig 6b, 7b, 8b). The intra-uterine fluid, echogenic free-floating structure and embryonic debris within the uterine lumen reduced over the period of examination (Fig 6b, 7b, 8b) and got reabsorbed within varying time indifferent cows. The cows with embryonic mortality show various ultrasonographic signs which include reduced intra-uterine allantoic fluid (Drews *et al*. 2012) and absence of embryo proper with an embryonic heartbeat (Flores *et al*. 2014). The lumen was filled with hyperechoic free-floating structures, representing embryonic debris (Drews *et al*. 2012; Flores *et al*. 2014), embryonic remnants with an ill-defined embryonic outline and distorted allanto-chorionic membrane (Lopez-Gatius and Garcia-Ispierto, 2010) which are in unison with our findings. The intra-uterine fluids reduced over time (Flores *et al*. 2014) and got reabsorbed by the maternal immune system.

Table 2: Comparative ultrasonography of pregnant, non-pregnant and with embryonic mortality in cows (Mean±SD; N=29).

Parameters	Day of examination	Pregnant (n=15)	Embryonic mortality (n=5)	Non-pregnant (n=9)
Corpus luteum	21	21.17±0.56^{ab}(18.15-25.05)	19.20±1.97^{ab}(15.30-26.05)	11.73±1.41^{c}(3.30-17.55)
size (mm)	28	20.47±0.58^{ab}(16.80-25.65)	21.43±1.06^{ab}(18.95-25.15)	4.35±2.08^{c}(0.00-13.90)
	35	21.95±0.74^{x}(15.85-26.45)	16.46±4.14^{y}(0.00-22.10)	-
	42	21.42±0.50^{a}(19.60-25.40)	13.55±3.71^{b}(0.00-19.70)	-
Corpus luteum	21	22.98±1.83^{a}(13.59-38.57)	15.30±3.75^{ax}(7.41-28.30)	5.20±1.91^{by}(0.00-16.00)
vascularity (%)	28	26.2±2.04(16.36-47.76)	17.21±5.85(5.00-36.95)	0.75±0.75(0.00-4.54)
	35	31.62±4.50^{a}(11.72-84.38)	9.47±3.78^{b}(0.00-21.73)	-
	42	32.06±3.93^{a}(15.17-58.62)	2.44±1.50^{b}(0.00-6.56)	-
Uterine horn	21	4.13±0.67(2.40-10.10)	2.09±0.86(0.00-21)	-
lumen (mm)	28	14.39±2.10^{a}(2.63-28.75)	2.12±0.66^{b}(0.00-28)	-
	35	22.78±2.64^{a}(12.30-52.00)	3.79±2.26^{b}(0.00-35)	-
	42	41.02±7.43^{a}(21.05-74.80)	4.37±2.72^{b}(0.00-42)	-
Embryo (mm)	21	-	-	-
	28	5.92±0.47(3.85-8.40)	-	-
	35	8.73±0.46(6.05-12.15)	-	-
	42	13.03±0.78(5.34-16.10)	-	-
Amniotic	21	-	-	-
Sac (mm)	28	9.56±0.62(7.30-12.35)	-	-
	35	12.39±0.61(7.90-17.00)	-	-
	42	21.63±1.01(17.00-27.75)	-	-

Note: Values with different superscripts (a, b, c) and (x, y) within a row for a particular parameter differ significantly at P<0.01 and P<0.05, respectively.

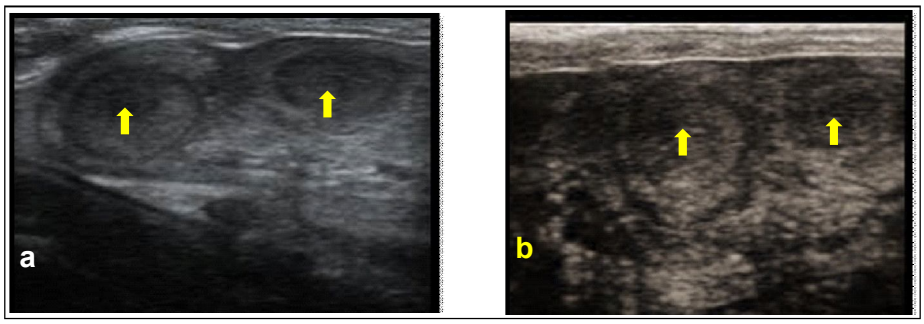

Fig 5: Ultrasonographic images of uterus and embryo in brightness mode on day 21;
[a] Pregnant - left uterine horn showing greater luminal size than right horn (arrow), [b] Embryonic mortality - uniform luminal diameter of both uterine horns.

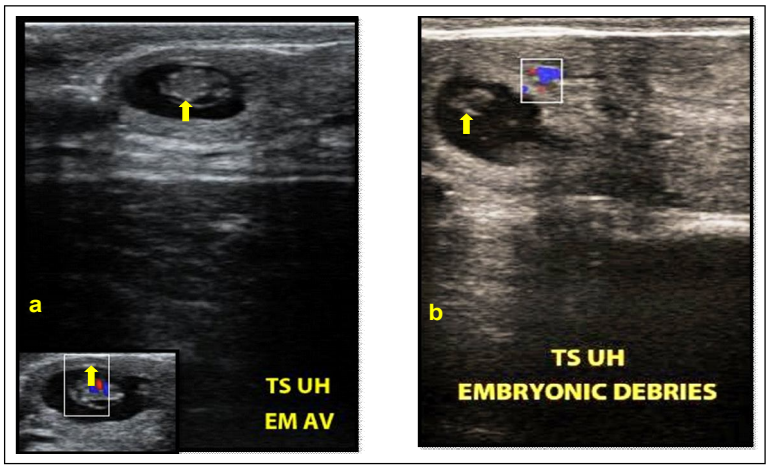

Fig 6: Ultrasonographic images of uterus and embryo in brightness and color Doppler mode on day 28; [a] Pregnant - uterine horn with embryo proper (embryo + amniotic sac) and clear uterine fluid along with positive color Doppler signal (heartbeat), [b] Embryonic mortality - ill-defined embryonic mass along with fragments of allanto-chorionic membrane within the uterine lumen.

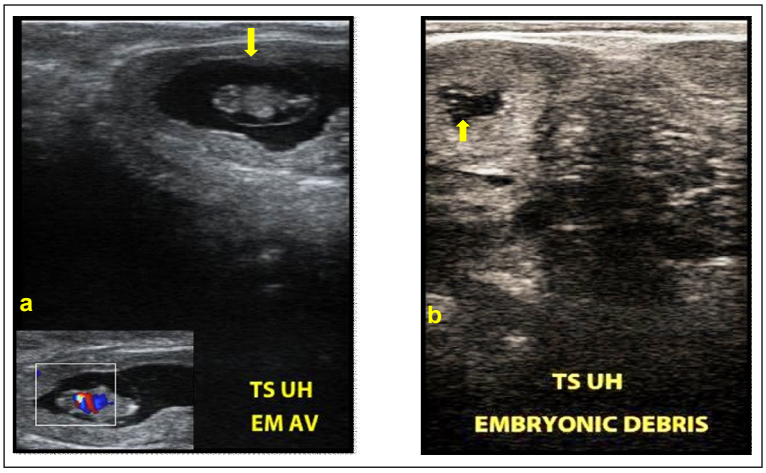

Fig 7: Ultrasonographic images of uterus and embryo in brightness and color Doppler mode on day 35; [a] Pregnant - embryo proper with placentomes and strong color Doppler signal [b] Embryonic mortality - reduction in intra-uterine fluid along with allantoic membrane fragments.

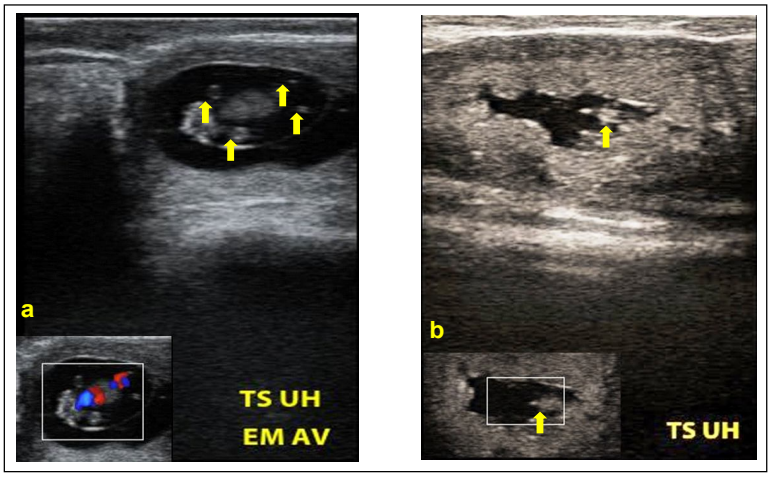

Fig 8: Ultrasonographic images of uterus and embryo in brightness and color Doppler mode on day 42; [a] Pregnant- embryo proper with strong color Doppler signal (heartbeat) and formation of fore-limb and hind-limb buds, [b] Embryonic mortality- embryonic remnant without positive color Doppler signals (heartbeat).

As a part of peroration, color Doppler ultrasonography has been very effective in the differential diagnosis of physiological (pregnant/non-pregnant) conditions as well as embryonic mortality based on CL size, CL vascularity, and embryonic characteristics.

ACKNOWLEDGEMENT

Authors would like to thank head of Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, CSKHPKV, Palampur for providing all the necessary facilities during the present study.

Disclosure statement

Authors declare no conflict of interest.

REFERENCES

Bansal, S.K., Bhagat, R.L., Sinha, A.K., Yadav, A.L. and Phadke, N.L. (2019). Factors affecting conception rate in AI bred cattle under field conditions of Bihar. Indian Journal of Animal Sciences. 89(1): 110-112.

Diskin, M.G. and Morris, D.G. (2008). Embryonic and early foetal losses in cattle and other ruminants. Reproduction in Domestic Animals. 43(2): 260-267.

Diskin, M.G., Waters, S.M., Parr, M.H. and Kenny, D.A. (2016). Pregnancy losses in cattle: Potential for improvement. Reproduction Fertility and Development. 28(1-2): 83-93.

Drews, B., Ringleb, J., Waurich, R., Hildebrandt, T.B., Schroder, K. and Roellig, K. (2012). Free blastocyst and implantation stages in the European brown hare: Correlation between ultrasound and histological data. Reproduction Fertility and Development. 25(6): 866-78.

Flores, L.E., Hildebrandt, T.B., Kuhl, A.A. and Drews, B. (2014). Early detection and staging of spontaneous embryo resorption by ultrasound biomicroscopy in murine pregnancy. Reproduction Biology Endocrinology. 12: 38.

Fricke, P.M., Ricci, A., Giordano, J.O. and Carvalho, P.D. (2016). Methods for and implementation of pregnancy diagnosis in dairy cows. Veterinary Clinics of North America Food Animal Practice. 32(1): 165-180.

Gabor, G., Kastelic, J.P., Abonyi-Toth, Z., Gabor, P., Endrodi, T. and Balogh, O.G. (2016). Pregnancy loss in dairy cattle: Relationship of ultrasound, blood pregnancy-specific protein B, progesterone and production variables. Reproduction in Domestic Animals. 51(4): 467-473.

Gatea, A.O., Smith, M.F. and Pohler, K.G. (2018). The ability to predict pregnancy loss in cattle with ELISAs that detect pregnancy associated glycoproteins is antibody dependent. Theriogenology. 108: 269-276.

Ginther, O.J. (2014). How ultrasound technologies have expanded and revolutionized research in reproduction in large animals. Theriogenology. 81(1): 112-125.

Gnemmi, G. (2004). Atlante di Ultrasonografia Ginecolo´gica Buiatrica. Ed. Le Point Veternaire, Milano, Italy, p. 127.

Gray, C.A., Bartol, F.F. and Tarleton, B.J. (2001). Developmental biology of uterine glands. Biology of Reproduction. 65(5): 1311-1323.

Hernandez, J.A., Risco, C.A., Lima, F.S. and Santos, J.E. (2012). Observed and expected combined effects of clinical mastitis and low body condition on pregnancy loss in dairy cows. Theriogenology. 77(1): 115-121.

Herzog, K., Voss, C. and Kastelic, J. (2011). Luteal blood flow increases during the first three weeks of pregnancy in lactating dairy cows. Theriogenology. 75(3): 549-554.

Jokubkiene, L., Sladkevicius, P., Rovas, L. and Valentin, L. (2006). Assessment of changes in volume and vascularity of the ovaries during the normal menstrual cycle using three-dimensional power Doppler ultrasound. Human Reproduction. 21(10): 2661-2668.

Lopez-Gatius, F. and Garcia-Ispierto, I. (2010). Ultrasound and endocrine findings that help to assess the risk of late embryo/early foetal loss by non-infectious cause in dairy cattle. Reproduction in Domestic Animals. 45(3): 15-24.

Luttgenau, J. and Bollwein, H. (2014). Evaluation of bovine luteal blood flow by using color Doppler ultrasonography. Reproductive Biology. 14(2): 103-109.

Pancarci, S.M., Ari, U.C., Atakisi, O., Gungor, O., Cigremis, Y. and Bollwein, H. (2012). Nitric oxide concentrations, estradiol-17β progesterone ratio in follicular fluid and COC quality with respect to perifollicular blood flow in cows. Animal Reproduction Science. 130(1-2): 9-15.

Pereira, M.H., Cooke, R.F., Alfieri, A.A. and Vasconcelos, J.L. (2013). Effects of vaccination against reproductive diseases on reproductive performance of lactating dairy cows submitted to AI. Animal Reproduction Science. 137(3-4): 156-162.

Pinaffi, F.L.V., Araujo, E.R., Silva, L.A. and Ginther, O.J. (2017). Color-Doppler signals of blood flow in the corpus luteum and vascular perfusion index for ovarian and uterine arteries during expansion of the allantochorion in *Bos taurus* heifers. Theriogenology. 102: 35-43.

Pohler, K.G., Green, J. and Moley, L.A. (2014). The Effect of Embryonic Size and Sire on Circulating Concentrations of Bovine Pregnancy Associated Glycoproteins (PAGs) in Beef Cows. In: Reprod. Fert.: Proc. 9th Int. Rumin. Reprod. Symp Context Products Ltd., Leicestershire, UK. p 565.

Pohler, K.G., Pereira, M.H.C. and Lopes, F.R. (2016). Circulating concentrations of bovine pregnancy- associated glycoproteins and late embryonic mortality in lactating dairy herds. Journal of Dairy Science. 99(2): 1584-1594.

Romano, J.E., Thompson, J.A., Kraemer, D.C., Westhusin, M.E, Tomaszweski, M.A. and Forrest, D.W. (2011). Effects of early pregnancy diagnosis by palpation per rectum on pregnancy loss in dairy cattle. Journal of American Veterinary Medical Association. 239(5): 668-673.

Romano, J.E. (2004). Early pregnancy diagnosis and embryo/fetal mortality in cattle. PhD thesis, Department of Animal Science, College of Agricultural and Life Sciences, Texas A and M University, College Station, Texas.

Scully, S., Butler, S.T., Kelly, A.K., Evans, A.C., Lonergan, P. and Crowe, M.A. (2014). Early pregnancy diagnosis on days 18 to 21 postinsemination using high-resolution imaging in lactating dairy cows. Journal of Dairy Science. 97(6): 3542-57.

Sharma, A., Mahajan, M., Singh, M. and Kumar, P. (2018). Ultrasonographic investigation of monochorionic diamniotic monozygotic twins in a cow. Veterinary Sciences: Research and Reviews. 4(2): 62-71.

Sharma, A., Singh, M., Abrol, A. and Soni, T. (2019a). Doppler sonography of uterine blood flow at mid-estrus during different degree of clinical endometritis in dairy cows. Reproduction in Domestic Animals. 54(9): 1274-1278.

Sharma, R., Singh, M., Kumar, P. and Sharma, A. (2019). Causes and therapeutic management of infertility in abandoned cows of Himachal Pradesh. Indian Journal of Animal Sciences. 89(9): 955-957.

Shirasuna, K., Matsumoto, H. and Kobayashi, E. (2012). Upregulation of interferon-stimulated genes and interleukin-10 in peripheral blood immune cells during early pregnancy in dairy cows. The Journal of Reproduction and Development. 58(1): 84-90.

Sreenan, J.M., Diskin, M.G. and Morris, D.G. (2001). Embryo survival rate in cattle: A major limitation to the achievement of high fertility. BSAP Occasional Publication. 26(1): 93-104.

Tamura, H., Takasaki, A., Taniguchi, K., Matsuoka, A., Shimamura, K. and Sugino, N. (2008). Changes in blood-flow impedance of the human corpus luteum throughout the lutealphase and during early pregnancy. Fertility and Sterility. 90(6): 2334-2339.

Whitlock, B.K. and Maxwell, H.S. (2008). Pregnancy- associated glycoproteins and pregnancy wastage in cattle. Theriogenology. 70(3): 550-559.