OVARIAN HAEMODYNAMICS AND FERTILITY IN CATTLE

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ABSTRACT

Ovarian vasculature is an important supportive component that ensures the fertility of an animal. Angiogenesis involves the formation of capillaries from pre-existing microvessels and therefore contributes to vascular remodelling and maturation. This process allows adequate nutritional and hormonal supply for ovarian follicle growth and corpus luteum (CL) formation. Colour-Doppler ultrasound has been used for studying vascular perfusion of ovarian structures and to analyse the blood flow parameters through Doppler spectrum. The ovarian follicle and CL are the major sites of active angiogenesis suggesting that a strong association between normal functionality of these structures and the network of blood vessels. Rapid pulse frequency of the peri-follicular blood flow is a prerequisite factor for induction of ovulation process. Sequential observation during an oestrous cycle revealed progressive increase in luteal blood flow with the increasing endocrine activity. There was an intense increase in luteal blood flow between days 15 – 17 of the oestrous cycle coinciding with the luteolysis. A deficient blood supply to the ovaries could disturb the follicular development, maturation and ovulation leading to various pathological conditions like anoestrus and cystic ovarian degeneration. More clinical research trials are required to develop suitable therapeutic protocols to modulate ovarian vascularity in order to enhance the fertility of cattle.

Keywords: Ovarian vascularity, angiogenesis, follicle, corpus luteum, fertility

Successful establishment of pregnancy requires a positive interaction between a healthy embryo and a receptive endometrium. One of the important supportive components of this interaction is adequate and effective vascular circulation. Vascular system influences the metabolic effects by transporting fluid, nutrients, oxygen, and waste material. In addition, the blood deliver hormones from other parts of the body, allowing them to perform their local actions. During the embryo development, blood vessels differentiate from endothelial precursors by a process called vasculogenesis. However, in adult body, to favour blood transport into growing tissues, hormones promote the proliferation of new blood vessels by a process called angiogenesis, also referred as neovascularisation.

Angiogenesis involves the formation of capillaries from pre-existing microvessels and therefore contributes to vascular remodelling and maturation (Clapp *et al*., 2009). It plays a pivotal function in a variety of normal and pathological conditions such as embryonic development, wound healing, tumour growth and metastasis (Folkman, 1995; Risau, 1997). In addition, several aspects of human and animal reproductive processes, such as physiological changes that occur in the utero-ovarian structures during various reproductive phases depend on angiogenesis (Acosta *et al*., 2003). This process occurs throughout follicular development, allowing adequate nutritional and hormonal supply for ovarian follicle growth and oocyte development, as well as corpus luteum (CL) formation (Fraser and Lunn, 2000).

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Doppler ultrasonography is routinely used in human gynaecological practice as the diagnostic gold standard in order to predict ovulation and fertilization outcomes (Chui *et al*., 1997; Borini *et al*., 2001). In the last two decades, there was a progressive interest in the study of utero-ovarian angiogenesis in veterinary field due to the association of this process with fertility of the farm animals especially cattle (Acosta *et al*., 2002, 2003; Miyamoto *et al*., 2005; Honnens *et al*., 2008; Herzog *et al*., 2010, Satheshkumar et al., 2014, 2017 and 2018).

ANATOMY OF OVARIAN VASCULATURE

The anatomy of the artery and its branches that supply an ovary in cattle has been described (Ginther and Del Campo, 1974; Lamond and Drost, 1974). The ovarian artery originates from the aorta and divides into a uterine branch and an ovarian branch about 6 cm away from the ovary. The ovarian branch divides in the ovarian pedicle into two or three branches, and each branch subdivides into two or three smaller branches. As a result, four to nine arteries enter the hilus of the ovary. The arterial branch that supplies the CL in cattle increases in diameter two- or threefold by the time the CL matures. Presumably, the same arterial branch previously supplied the preovulatory follicle.

Understanding blood flow parameters

Colour-Doppler ultrasound has been used for studying vascular perfusion of the dominant follicle (DF), CL and uterine structures. Colour-Doppler scanners respond to the movement and direction of flow of red cells in arteries and arterioles, and can impose colour signals of blood flow on a B-mode gray-scale image (Ginther and Utt, 2004). The Doppler waveform, known as 'spectral Doppler', represents changes in blood flow during one cardiac cycle. Doppler colour-flow mapping and spectral analyses can be done by three ways.

· ***Qualitative method***: Depends mainly on analysis of wave characteristics as the presence or absence of end diastolic blood flow and its relation to the previous and next peak systolic blood flow. It also concerns the wave characteristics as above or under the baseline, in addition to increased and/or decreased end diastolic velocity of blood flow, either physiological or pathological.

· ***Semi-quantitative***: Measurements of the Doppler indices viz., pulsatility index (PI) and resistance index (RI), include calculations of peak systolic velocity (PSV), End diastolic velocity (EDV) and the time averaged maximum velocity (TAMV) values over the time of the cardiac cycle (Fig.1). These Doppler indices provide important information to enable the researcher or the clinician to understand the extent of vascular perfusion.

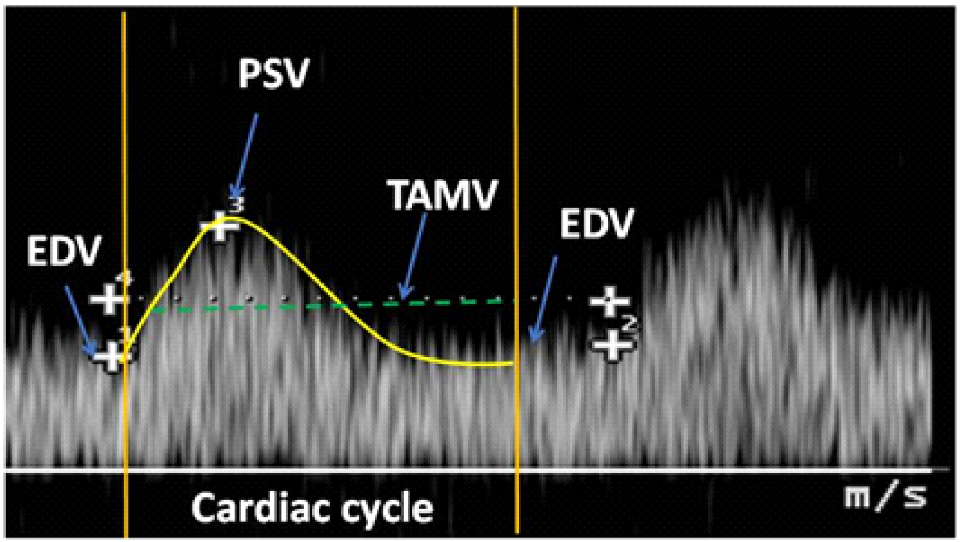

Fig. 1: Measurement of various velocity parameters in a cardiac cycle

Flow velocity waveforms that were obtained from those vessels were used for spectral analysis. The PSV and EDV was recorded from the flow velocity waveform. Blood flow impedance was expressed as the RI and the PI and were calculated from curves fitted to the flow velocity waveform over three cycles according to the formula:

RI = (PSV “ EDV) / PSV
PI = (PSV “ EDV) / TAMV

- ***Quantitative***: Analysis includes evaluation of diameter of the target blood vessel and blood flow volume.

Other colour Doppler studies involved the degree of vascular perfusion by assessing the percentage of follicle circumference with colour signals.

ROLE OF BLOOD FLOW ON OVARIAN PHYSIO LOGY

Ovarian angiogenesis

Ovarian angiogenesis plays an important role in the sequence of events leading to the development of the ovarian follicle and the formation of the CL. The poor oocyte quality due to abnormal ovarian perfusion and decreased ovarian reserve can play an important role in the pathogenesis of infertility (Zebitay *et al.*, 2015).

FOLLICULOGENESIS IN RELATION TO BLOOD FLOW

The ovarian follicle is a site of active angiogenesis. The follicles upto the small preantral stage, follicles are avascular and angiogenesis is initiated at this time and continues throughout follicular growth (Wulff *et al*., 2001). The vascular network in the follicle is confined to the thecal layer and thecal capillaries do not penetrate the basement membrane so that the granulosa compartment remains avascular until the basement membrane breaks down at ovulation. The ovulatory follicle (OF) had a high degree of vascularity compared with subordinate follicles (Zeleznik *et al.*, 1981) and regression of the thecal vascular network was observed in anovulatory follicles and during atresia (Jiang et al., 2003). These findings suggest a strong association between follicular development and the network of follicular blood vessels.

FOLLICULAR BLOOD FLOW AND OVULATION

Normal ovulation process is a prime factor that determines the fertility of an animal. The mediatory role of vasoactive substances released during luteinizing hormone (LH) stimulation has been suggested to be play an important role in the ovulation process (Acosta *et al*., 2003). Subsequent reports also related the importance of pre-ovulatory follicular vascularity to conception rate and fertility (Siddiqui *et al*., 2009; Pancarci *et al*., 2012; Varughese *et al*., 2017). The intensity of peri-follicular blood flow increased as the follicle approached ovulation which was evident by enriched color Doppler signals covering over 70 per cent of the OF circumference (Satheshkumar, 2018). This increase in the intensity of peri-follicular Doppler signals indicated active neovascularization process and increasing arteriolar network encasing the OFs (Ginther, 2007). Since the supply of hormones, nutrients and oxygen are ensured by adequate blood supply to the follicles, the strong association between angiogenic process and the selection of ovulatory follicle has been confirmed.

The relationship between the pulse frequency of the peri-follicular blood flow and the timing of ovulation in crossbred cattle has been documented (Satheshkumar, 2018). A new parameter was developed by measuring the linear distance between two end diastolic points of a spectral wave, and coined the term as Doppler pulse duration (DPD). It was measured in milliseconds (ms). Decreased values of DPD are indicative of rapid pulse frequency and vice versa. It was clearly observed that the OFs with a lower DPD (< 900 ms) on the day of oestrus ovulated within the normal duration. On the contrary, OFs with higher mean DPD value required an extended time for ovulation (Fig.2). Thus it was evident that rapid pulse frequency of the peri-follicular blood flow is a prerequisite factor for induction of ovulation process.

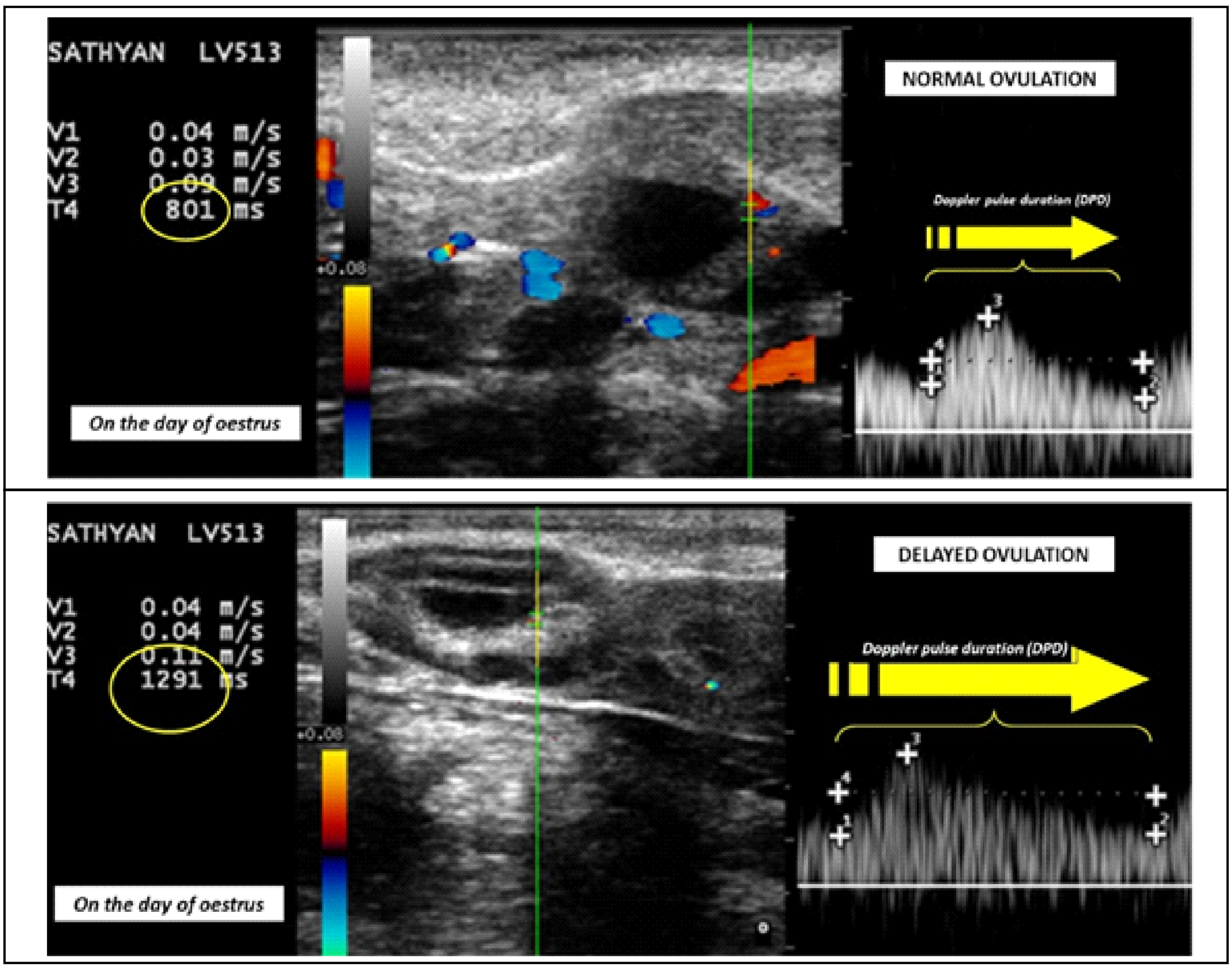

Fig.2: Doppler Pulse duration (DPD) of perifollicular blood flow of ovulatory follicle

Studies recorded lower PI values in the preovulatory follicular blood flow of women (Anteby *et al.*, 1996), mares (Palmer *et al.*, 2006), heifers(Siddiqui *et al.*, 2009) and crossbred cows(Satheshkumar, 2018) respectively. They attributed the phenomenon to angiogenesis, greater vascular perfusion and vasodilation. A positive correlation between capillary angiogenesis and intrafollicular concentration of oestradiol (E_2) in bovine antral follicles has been reported (Jiang *et al.*, 2003) Furthermore, several studies have reported that E_2causes a rapid dilation of blood vessels by increasing the bioavailability of nitric oxide (NO) concentrations in follicular fluid (Bonello et al., 1996; Acosta *et al.*, 2003; Pancarci *et al.*, 2012). Scanning electron microscopic observations of ovarian corrosion casts by Macchiarelli *et al*. (2006) confirmed the fact that capillaries of preovulatory follicles were much dilated than that of earlier developing follicles.Thus, the decreased DPD and PI values on the day of oestrus are positive indicators of normal ovulation process and any deviations in these blood flow parameters delayed or ceased the phenomenon. The finding is a valuable diagnostic parameter and sheds light for future studies involving regulation of follicular vasodilation to hasten the ovulation process in cattle.

VASCULAR PERFUSION IN CL

Fertility of any mammalian species is closely related to their luteal activity. CL is a dynamic structure which exhibits regular periods of growth, development and regression throughout the fertile phases of reproduction. The elevated levels of pro-angiogenic factors (VEGF and FGF2) stimulate angiogenesis after ovulation, during luteinisation and throughout the development of the CL during mid luteal phase (Zalman *et al.*, 2012)

The normal luteal blood flow changes throughout the untreated cycle were sequentially documented in crossbred cattle (Satheshkumar *et al.*, 2014). Blood flow could be observed as sparse colour Doppler signals around the periphery of the luteal tissue from Day 3, which increased gradually and covered more than 70% of the luteal circumference during the mid-luteal phase (Day 9 – 10). The progressive increase in the luteal blood flow could be attributed to increase in the endocrine activity of the structure (Kaya *et al.*, 2017). The vascularity enables the luteal cells to obtain the oxygen, nutrients and hormone precursors that are indispensable to support its endocrine activity (Fraser and Wullf, 2003). Sequential observations revealed that there was an intense increase in blood flow between Day 15 – 17 of the oestrous cycle, which could be appreciated by penetration of colour Doppler signals deep into the luteal parenchyma (Satheshkumar *et al.*, 2014). The intense increase in blood flow has been correlated to the pulsatile release of PG from the uterus as indicated by drastic increase in the plasma PGFM concentration during the period (Miyamoto *et al.*, 2006). Thus it could be concluded that the increased vascularisation of CL during Day 15 – 17 of the cycle was an indication of luteal response to PG which was followed by luteal regression. After that period, the blood flow signals decreased and restricted to the periphery as the CL regressed towards the final stages of the cycle (Day 19 – 21).

VASCULAR PERFUSION DURING PROGRAMMED LUTEOLYSIS

Luteolysisis the key event in the normal luteal life span that determines the cyclicity of the animals. Programmed induction of complete luteolysis is possible by administration of exogenous PG during the mid-luteal phase, but the CL is refractory to PG treatment during the early-luteal phase of the oestrous cycle (Beal *et al.*, 1980). The non-responsiveness of early CL was suggested to be due to luteal insensitivity or insufficient numbers of PG receptors (Duchens et al., 1994). PG exerts its luteolytic effects by binding to its plasma membrane receptors (Sakamoto *et al.*, 1994). Subsequently, apoptosis of luteal cells is caused by reduction in blood flow and hypoxia induced inhibition of steroidogenic enzymes. Although these events contribute to the later stages of luteolysis, little is known about the cascade of physiological events initiating the luteolytic process. Acosta *et al*. (2002) and Shrestha and Ginther (2011) observed an acute increase in luteal blood flow in response to a conventional dose of PG administered in both the early-luteal phase (Day 3) and the mid-luteal phase (Day10). However, Satheshkumar *et al*. (2014) observed that the intensified colour Doppler signals in response to exogenous PG was restricted only to the periphery of the CL in early stages of CL development, but vascular perfusion deeply radiated spreading over 80% of the luteal parenchyma by 2 h post PG in mid-luteal phase (Fig.3). The PG induced endothelial Nitric Oxide Synthase – Nitric oxide (eNOS-NO) system would have dilated the microvascular bed within the mid luteal CL, resulting in free flow of blood deep into the luteal parenchyma. This vascular perfusion would have enabled the luteolysin to reach the deeper luteal cells and initiated the luteolytic process in the mature mid cycle CL, rather than the immature early CL.

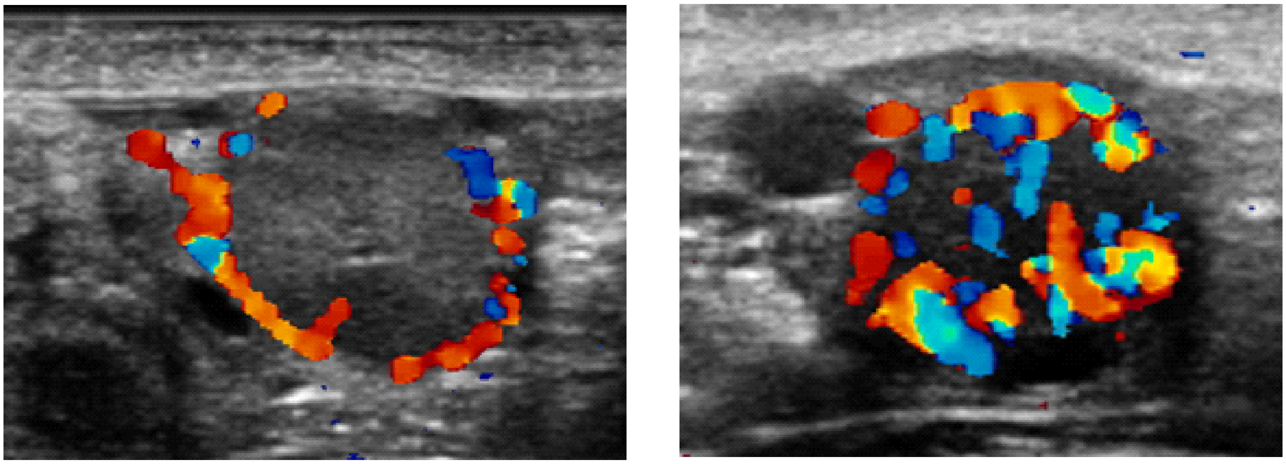

Fig.3: Vascular perfusion pattern in CL of different stages in response to PG

In natural cycle, gradual increase in blood flow could be observed for an extended period of 2-3 days before spontaneous luteolysis, but in PG treated cycles the vascular perfusion episode was acute. On the whole, it could be concluded that both spontaneous and induced luteolysis are characterized by an initial increase in luteal vascular perfusion.

ROLE OF DISTURBED BLOOD FLOW ON OVARIAN PATHOLOGY

The advent of colour doppler imaging facilities provides new insights into infertility disorders. Ovarian vascular perfusion plays a major role in delivering nutrients, hormones and growth factors to the highly vascularised ovarian structures (Gaytan *et al*., 1999). A deficient blood supply to the ovaries could disturb the follicular development, maturation and ovulation leading to various pathological conditions like anoestrus, cystic ovarian degeneration (COD),etc. The initiation of the physiopathological angiogenic response, known as the ‘angiogenic switch’, depends on the dynamic balance between exogenous or endogenous stimuli (pro-angiogenic factors) and inhibitors (anti-angiogenic factors) acting in the immediate environment of endothelial cells (Hanahan and Folkman, 1996; Distler *et al*., 2002). The disruption of such equilibrium can favour the emergence of new vessel formation or lead to vessel quiescence or regression (Hanahan and Folkman, 1996).

ANOESTRUM

Post-partum anoestrus,a major reproductive disorder in crossbred cattle, occurs as a result of multiple factors including nutrition (Kumar *et al*., 2014). Neovascularisation is crucial for antral follicle growth, dominance and pre-ovulatory development since studies have shown that anti-angiogenic compounds reduced the thecal vascularity and consequently severely compromised follicular development (Wulff *et al*., 2002; Fraser and Duncan, 2009).

Satheshkumar *et al*. (2017) studied the differences in blood flow parameters of ovarian artery in oestrus and anoestrus cattle. The study revealed that the blood velocity parameters were lower but the PI, RI and DPD indices were higher in the anoestrus cattle. Thus, low velocity, increased flow resistance (indicated by high PI and RI) and slow pulsation (indicated by high DPD) of the blood flow in ovarian artery of anoestrus animals can be affecting the vascular perfusion of follicles. In brief, ipsi-lateral ovarian artery blood flow is significantly correlated to follicular vascular perfusion and altered blood flow parameters of ovarian artery proved to be one of the causative factors for poor follicular development in anoestrus cattle.

CYSTIC OVARIAN DEGENERATION

Cystic ovarian degeneration (COD) is an important ovarian dysfunction and a major cause of reproductive failure in dairy cattle. Ovarian follicular cyst is a consequence of a mature follicle that fails to ovulate at the appointed time during the oestrous cycle. The incidence of COD among dairy cattle ranges from five to 30 per cent (Ortega *et al*., 2016). Both follicular growth and follicular atresia are regulated by the extent and type of vascular development (Hazzard and Stouffer, 2000) and an alteration in this process can result either in a disruption of the physiological state or in the occurrence of pathological conditions (Abulafia and Sherer, 2000). Diaz *et al*. (2019) experimentally induced persistence of follicle, simulating the COD, and found that the percentage of the blood irrigation area in the wall of persistent follicles was less than those observed in the wall of normal preovulatory follicles. The effectiveness of the hormonal treatment of COD depends on the presence of a functional luteinizing hormone receptor

(LHR) along with an increase in angiogenic factors and an increase in follicular blood flow. As the persistence time progresses, the alterations in follicular blood flow together with changes in the development and functions of follicular cells could partially explain the variability in the ovarian blood flow responses after hormonal treatment in animals with spontaneous COD (Rauch *et al.*, 2008; Marelli *et al.*, 2014). The evaluation of the intensity of blood flow with the use of power Doppler could provide information about the viability of persistent or cystic follicles, and could contribute to a more precise diagnosis and therapeutic approaches in assessing these pathologies.

CONCLUSION

The ovarian vascularity and angiogenesis is a complex process. The intense angiogenesis and increased permeability of blood vessels are associated with follicular development, ovulation and subsequent formation of the corpus luteum. The expression of a range of key angiogenic factors vary according to the stage of the oestrous cycle. The disturbance of this balance may result in a disrupted physiologic state ultimately leading to infertility. Thusthe ovarian haemodynamics has been considered to play a critical role in the fertility of the dairy cattle. Future studies are warranted on positive modulation or regulation of ovarian vascularity toward enhancing the fertility of cattle.

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