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A Potential Use of Doppler Ultrasound as a Tool for Reproductive Studies in Cows

A thesis submitted by

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Abstract

The present study aimed to use color and spectral Doppler ultrasound to study both follicular, luteal blood flow vascularization areas and ovarian and uterine arteries Doppler indices after single and multiple ovulations and after induction of ovulation using either Ovsynch or CIDR evaluated. Nitric oxide and ovarian hormones were estimated in blood serum. Results of the non-induced ovulation showed that both days and phases of the oestrus cycle influenced (P=0.0001) the follicular dynamic, the luteal dynamic, the ovarian and uterine hemodynamic. The ovulatory wave and the mid-luteal non-ovulatory wave had expanding numbers and the diameters of small, medium and large follicles. Though area, antral area, vascularization area of the (ovulatory folcile) OF ascended from day -4 to the day of ovulation (day 0), but the percent of the vascularization area and the granulose layer increased till day -3. The CL diameter and luteal vascularization area increased till day 13, and 15, but luteal % of vascularization area ascended (P=0.0001) from days 1 to 4 and declined from days 9 to 13. Both (resistance index)RI and(pulstility index) PI of the ipsilateral ovarian artery were lower than the contralateral one, but both obtained high values during the follicular phase. A linear increase (P=0.0001) of uterine horns vascularization area, both ovarian and uterine arteries diameters, (peak systolic velocity)PSV and (end diastolic velocity) EDV from follicular to late luteal phases was accompanied by a linear decrease of their PI and RI. Ovsynch ipsi-lateral Ov.A and Ut.A blood flows were lower ($P<0.05$) than the spontaneous ones as expressed by lower diameters, PSV, EDV, BFV and S/D accompanied higher PI and RI. The ovsynch contra-lateral Ov.A had higher ($P<0.01$) PI but lower TAMV during follicular and early luteal phases. The ovsynch contralateral Ut.A had lower ($P<0.05$) PSV and EDV during follicular and late-luteal phases. Ovsynch ovulation had low E_2, NO and high glucose but the late luteal had high ($P<0.001$) P4 and glucose but low E2 compared to the spontaneous ovulations. The right ovarian artery had higher blood flow parameters and Doppler indices after ovulation and the parameters of the right uterine artery increased after CIDR insertion reaching a maximum value on day of prostaglandin injection then descended reaching low values on day 2, while the parameters of the left uterine artery decreased sharply from day -7 and reached minimum level on day 18. Estradiol increased but progesterone decreased from day -7 till day of ovulation. P_4 reached maximum values on day 12 and decreased after that till day 18.Nitric oxide metabolites showed one peak on day -1 and another on day 6 and 8. The results of superovulation revealed that the ovulatory wave (days -5 to 0), and the non ovulatory wave (days 4 to 9) after ovulation were associated with increased numbers and diameters of small (≤0.5cm), medium (>0.5-≤0.1cm), and large (>1.0cm) follicles and from ≥13 follicles, five follicles ovulated from both ovaries. The PSV of uterine arteries decreased while that of ovarian arteries increased from day -4 to Day 0. Both ovarian arteries diameter, RI, PSV, EDV and S/D positively correlated ($P<0.0001$), but their PI negatively correlated ($P<0.0001$). The uterine arteries PI, RI, PSV, EDV, TAMV and S/D negatively correlated ($P<0.0001$) but their diameters positively correlated. Estradiol increased but progesterone decreased from day -5 till Day 0. After ovulation, P_4 reached maximum values on day 9 and started to decrease till day 19.Nitric oxide showed one peak on day -3 and another one from day 3 to day 9. In conclusion, the ovarian and uterine blood flows vary according to the estrous day, estrous phase, the ovulating ovary, ovulatory follicle growth and corpus luteum developmental stage. In addition to, the decrease of the follicle blood flow may decrease the follicle quality and the decrease of the luteal vascularization and their association with lowered uterine blood flow may disturb the maintenance of the embryo when the animals undergo timed insemination following the ovsynch protocol. As well as there was the same pattern of blood flow in both ovarian arteries and different pattern of blood flow in both uterine arteries and depended on pre- or post-ovulation. Finally, in superovulation, the blood flow of ovarian arteries is different from uterine arteries vascular perfusion.

Keywords: Doppler, ovarian artery, uterine artery, cows, eCG, ovsynch, CIDR

Summary

This present study focused on evaluation of the normal blood flow dynamics and effect of the days and phases of the estrous cycle on follicular dynamics, preovulatory follicle vascularization, luteal vascularization, and uterine horns vascularization and relating them to the ovarian and uterine blood flow.Also the current work focused on evaluation the blood flow waveform of the dominant follicle, the developing corpus luteum and the uterus associated with uterine and ovarian arterial blood and volume flows in association with the fluctuations of progesterone, estradiol, glucose, and nitric oxide in Friesian cows treated with Ovsynch and CIDR synchronization programs,in addition to superovulation by eCG in comparison to their previous spontaneous ovulations.The current study used adult, healthy cycling Friesian cows of 3-5 years old, 3.5 BCS, 420 ± 20 kg body weight and animals were examined (n=8) day after day during 3 normal successive cycle ,during treatment by Ovsynch and CIDR programs and blood samples were taken from each cow before examination, After removal of the CIDR devices, cows were examined each other day with Doppler ultrasound to determine the first and second ovulations (Day 0). On day 10 after the second spontaneous ovulation (Day -5) cows received i.m. injections (1500 IU) of equine chorionic gonadotropin .Blood flow areas were measured for all follicles, corpus luteum and uterus using Doppler ultrasonography, as well as ipsilateral and contralateral ovarian and uterine arteries were also measured with colored and pulsed Doppler giving spectral graph. Statistical analysis was done to study the effect of days and phases on ovarian follicles growth and vascularization, corpus luteum growth and vascularization. Pearson correlation coefficients was also processed to correlate the ovarian and uterine arteries diameters (CS), RI, PI, PSV, EDV,TAMV ,S/D and their mean.

Our results showed:

1. **Dynamics of uterine and ovarian arteries flow velocity waveform and follicular changes during the normal estrous cycle**

It was noticed that both days and phases of the normal estrous cycle influenced the follicular dynamic, the luteal hemodynamics, the ovarian and uterine hemodynamic. The ovulatory wave and the mid-luteal non-ovulatory wave had expanding numbers and the diameters of small, medium and large follicles. Though area, antral area, vascularization area of the ovulating follicles ascended from day -4 to the day of ovulation, but the percent of its vascularization area and that of granulose layer increased till day -3. The Corpus luteum diameter increase till day 15, and its vascularization area increased till day 13, but its % of vascularization area ascended from days 1-4 and declined from days 9-13. Both RI and PI of the ipsilateral ovarian artery were lower than the contralateral one; but, both obtained high values during the follicular phase. A linear increase of uterine horns vascularization area and both ovarian and uterine arteries diameters, PSV and EDV from follicular to late luteal phases

accompanied a linear decrease of their PI and RI.It was concluded that the ovarian and uterine blood flows waveform vary according to the estrous day, the estrous phase, the ovulating ovary, ovulatory follicle growth and corpus luteum developmental stage. As well as the blood flow to the ipsilaterial ovarian and uterine arteries is pronounced than the contralateral ones

2. **Effect of Synchronization Program (ovsynch)on Follicular and Luteal Vascularization Percentages, Ovarian and Uterine Arteries Blood Flow Volumes in Friesian Cows**

It was showed that the existing ovulating follicle area, antrum area and color area descended and associated the descent of the regressing CL diameter, area and color area from day -11 (1st GnRH) till day -5 then another ovulating follicle ascended from day -5 to ovulation (day 0). Ovsynch pre-ovulatory phase had more small, medium and total follicles. Though ovulating follicle tended to have large area, antrum area but % of colored area was lower than the spontaneous one. After ovulation, the ovsynch CL area was higher but the % of color area decreased dramatically and this decrease became significant during the mid- and late-luteal phases. Ovsynch ipsi-lateral Ov.A and Ut.A blood flows were lower than the spontaneous ones as expressed by lower diameters, PSV, EDV, BFV and S/D accompanied higher PI and RI. The ovsynch contra-lateral Ov.A had higher PI but lower TAMV during follicular and early luteal phases. The ovsynch contralateral Ut.A had lower PSV and EDV during follicular and late-luteal phases. Ovsynch ovulation had lower estradiol, nitric oxide metabolites and higher glucose but the late luteal had higher progesterone and glucose but lower estradiol compared to the spontaneous ovulations.In conclusion, the decrease of the follicle blood flow may decrease the follicle quality and the decrease of the luteal vascularization and their association with lowered uterine blood flow may disturb the maintenance of the embryo when the animals undergo timed insemination following the ovsynch protocol.

3. **Effect of controlled internal drug release device (CIDR) on ovarian and uterine arteries blood flow velocities and follicular dynamics in relation to hormonal changes and nitric oxide in cows**

It was noticed that both ovulating follicles and antrum area/pixel ascended throughout CIDR insertion from day -3 to Day 0. The developed corpus luteum (CL) had the same

ascending pattern of their area and color area after ovulation. The right ovarian artery had higher blood flow parameters and Doppler indices after ovulation compared to those before ovulation. The parameters of the right uterine artery increased after CIDR insertion reaching a maximum value on day of prostaglandin injection then descended reaching low values on day 2, while the parameters of the left uterine artery decreased sharply from day -7 and reached minimum level on day 18. Estradiol increased but progesterone decreased from day -7 till Day of ovulation. Progesterone reached maximum values on day 12 and decreased after that till day 18. While nitric oxide metabolites showed one peak on day -1 and another on day 6 and 8. It was concluded that both ovarian arteries blood flow and right uterine artery vascular perfusion are higher from day 2 after ovulation .The mechanism of NO vasodilatation during follicle development on ovarian arteries is mediated by the increased estrogen, and on uterine arteries after ovulation are mediated by increased progesterone production.

4. **Ovarian and uterine arteries blood flow velocities waveform, hormones and nitric oxide in relation to ovulation in cows superstimulated with equine chorionic gonadotropin and luteolysis induction 10 and 17 days after ovulation**

It was showed that from ≥13 follicles, five follicles ovulated from both ovaries. The ovulated follicles increased antrum color area and color area % till day -1. The developed corpora lutea attained similar diameter, area, color area, and color area % from day 2 till day 15. The PSV of uterine arteries decreased while that of ovarian arteries increased from day -4 to day 0. Both ovarian arteries diameter, RI, PSV, EDV and S/D positively correlated, but their PI negatively correlated. The uterine arteries PI, RI, PSV, EDV, TAMV and S/D negatively correlated but their diameters positively correlated. Estradiol increased but progesterone decreased from day -5 till day 0. After ovulation, P4 reached maximum values on day 9 and started to decrease till day 19.Nitric oxide showed one peak on day -3 and another one from day 3 to day 9. The ovarian blood flow is higher during multiple follicle development but the uterine blood flow is higher during corpora lutea development. The vasodilatation mechanisms of Nitric oxide are mediated by increased estrogen. It was concluded that corpora lutea developed after ovarian stimulation required more than one dose of the normal luteolytic dose for the induction of luteolysis