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Studies on mare infertility using Doppler ultrasound

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ABSTRACT

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Abstract

Mares (N=100) of 4-18 years old and of body weight 400-600 kg belonged to Police horse studs (El-Basateen, Tora, Police Academy) and some private farms were examined with Doppler ultrasound to monitor the blood flow of the reproductive system of fertile mares as well as some infertility problems. Blood flow to anovulatory follicle, ovarian inactivity, granulosa cell tumor (GCT) was compared to the dominant follicular and luteal blood flow. Blood flow of the uteri with cysts during estrus and diestrus and abnormal uterine fluids (endometritis) during estrus and during diestrus were compared to normal uterine vascularization during estrus and diestrus. Granulosa cell tumor had the highest red color blood flow and total color blood flow area. Blue color blood flow area of the corpus luteum was high compared to the dominant follicle and inactive ovary. Uterus with endometrial cyst and abnormal uterine fluids during estrus of infertile mares had high red color blood flow, blue color blood flow, and power blood flow areas compared to the normal uterus during estrus. It could be concluded that Doppler ultrasound could distinguish between normal follicle, anovulatory follicle, functional corpus luteum and inactive ovaries. Moreover, the increased uterine blood flow area of mares with abnormal fluids in their uteri (endometritis) and uterine cysts could distinguish from the normal uterine blood flow during estrus.

Keywords: anovulatory follicle, granulosa cell tumor, uterine cyst, endometritis, anestrum, mares

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LIST OF ABBREVIATIONS

3D-ISM	Three-dimensional internal structure microscopy
AI	Artificial insemination
BFV	Blood flow volume
CH	Corpus hemorrhagicum
CL	Corpora luteum
DT	Dinoprost tromethamine
EDV	End diastolic velocity
FSH	Follicular stimulating hormone
GCT	Granulosa cell tumor
GNRH	Gonadotropin releasing hormone
ir-INH	Immunoreactive inhibin
LBF	luteal blood flow
LH	Luteinizing hormone
PI	pulsatility index
PGF2 α	Prostaglandin F2 alpha
PSV	Peak systolic velocity
RI	Resistance index
TAMV	Time-averaged maximum velocity
UA	Uterine artery
US	Ultrasound
VEGF-A	Vascular endothelial growth factor A

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ARABIC SUMMARY……. ١

Introduction

Until some decades ago, the horse was the most important domestic animal being used in all types of farming, transport, war, and as a source of meat when necessary **(Ginther, 1992).** The many advances that have occurred in the developed countries in farm mechanization, especially from the time of the Second World War, have rendered the horse obsolete in most of its previous activities. Today the horse used in sport and recreation.

Reproduction is a global concern in the equine industry and unlike other livestock animals horses have been selected based on factors (e.g. performance, lineage) other than reproductive efficiency. Pregnancy rates in mares are generally viewed as poor when compared to other livestock animals. Mares have been reported to suffer from up to 30% pregnancy loss after confirmation of pregnancy and thought to be due to many factors, which include poor nutrition (**McKinnon, 2009**), And added that poor reproductive efficiency and high pregnancy losses in mares result in a significant loss of potential revenue and monetary expenditure, with an increased investment of time and labor to horse owners worldwide.

From the first application of traditional ultrasound in equine reproductive management (**Ginther and Pierson, 1984; Pierson and Ginther, 1985a,b)**, till now, ultrasound technology provides a non-invasive form of visual access to the reproductive organs of the equine to evaluate normal, morphological changes and to investigate certain pathological processes .

Doppler ultrasound is an emerging technology that has the potential to increase the diagnostic, monitoring and predictive capabilities of equine researchers. A Doppler ultrasound study is usually part of a vascular ultrasound examination (**Ginther and Utt, 2004).**

Doppler ultrasonography is a non-invasive real-time pulse-wave technique recently used for the transrectal study of the reproductive system hemodynamics in large animals **(Ferreira, Ignácio, Meira, 2011).** Color and power Doppler ultrasonography have been used to study the uterine hemodynamic of pregnant and non-pregnant mares (**Bollwein, Weber, Woschée and Stolla, 2004; Ginther, 2007;**

Ferreira, Gastal, and Ginther, 2008). Color Doppler ultrasonography (**Maulik, 1995**) has been used for noninvasive hemodynamic studies of preovulatory follicles in mares (**Abdelnaby and Abo El-Maaty, 2017a**).

In cows, transrectal color Doppler ultrasonography demonstrated a clear difference in the vascularity of the wall of preovulatory follicles compared with that of anovulatory (atretic) follicles (**Acosta, Hayashi, Ohtani, and Miyamoto, 2003**).

The color Doppler approach has a potential for investigating the vasculature of seasonal anovulatory dominant follicles in mares and in identifying dominant follicles that will fail to ovulate. The presence of anovulatory hemorrhagic follicles during the estrous cycle of mares causes financial impacts, slowing conception and increasing the number of services per pregnancy (**Lima, Costa, Alvarenga, Martins, 2015**). The future anovulatory dominant-sized follicle is characterized by LH deficiency and reduced blood flow area (**Acosta, Beg, and Ginther, 2004**). Hemorrhagic anovulatory follicle is the most common pathological anovulatory condition in the mare (**Martínez-Boví and Cuervo-Arango, 2016**).

Age of the mare has no effect on diameter of the preovulatory follicle, or B-mode echotexture or color-Doppler signals of blood flow in the follicular wall (**Ginther, Gastal, Gastal, Jacob and Beg, 2009**).

Granulosa-theca cell tumor (GCT) is one of the most common ovarian neoplasms in mares, with the age range at diagnosis commonly between 5 and 9 years (**Daels and Hughes, 1993)**.

Color Doppler monitoring offered valuable information on corpus luteum (CL) activity and vascularization in the mare and that the technique was a useful noninvasive tool for examining luteal blood flow (**Bollwein, Mayer, Weber, and Stolla, 2002**). Luteal vascularization plays an important role in the physiology of the CL (**Galvão, Ferreira-Dias, Skarzynski, 2013**). Intense angiogenesis occurs during luteinization in equine (**Ferreira-Dias, Bravo, Mateus, Redmer, and Medeiros, 2006**).

Therefore, the present study aimed to:

1- Checking the accuracy with the application of Doppler ultrasound for the diagnosis of reproductive infertility in mares through monitoring ovarian structures vascularization during ovulation failure compared to the normal preovulatory follicle.
2- Monitoring ovarian vascularization during anestrum and granulosa- cell tumours (GCT's).

3- Monitoring changes in uterine blood supply during endometritis and abnormal uterus post-mating.

4- Monitoring changes in uterine blood supply with uterine cyst compared to uterine vascularization during normal estrous phase and diestrous phase.

Review of Literature

Several decades ago, new techniques have been introduced in animal reproduction such as artificial insemination, synchronization of the estrous cycle, superovulation, embryo transfer, in vitro fertilization, and diagnostic ultrasonography **(Pierson, and Ginther, 1985a)**. Spatial compounding, harmonic imaging, multidimensional and extended field-of-view images are of many advancements in ultrasound technology. In addition, the improvements in transducer capabilities enhanced the ultrasonographic examination of the equine. The improvements in software and hardware capabilities helped overcome artifacts, improved image quality, and allowed better documentation of the examination for follow-up studies. Moreover, smaller and more portable machines produced better images is ideal for the ambulatory practice setting (**Neelisand Roberts, 2012).**They added that Ultrasound (US) scanning gives theability to the clinician to visualize various functional structures of the reproductive tract. Moreover, US had not only improved the timing of insemination and pregnancy detection, but it had also allowed understanding of follicular status, embryonic characteristics, uterine pathology, monitoring the postpartum mare and detection of ovulation

1. Ovarian ultrasonography and blood flow

Ultrasonography had rapidly become established as one of the principal imaging techniques used in veterinary practice because it is a non-invasive technique, so it had a considerable advantage over other diagnostic methods. It allowed the clinician to obtain instant information about a wide range of body systems and in some cases the dynamic function of organs could be assessed. Equine reproduction gained a significant diagnostic tool. In contrast to the conventional methods, ultrasonography permitted a much earlier and more accurate diagnosis of pregnancy and provided relevant practical information about normal and pathological conditions of the reproductive organs **(Ginther, 1995a)**.

Several infertility problems are encountered in mares. Every year, many mares fail to become pregnant, despite several attempts using semen from a fertile stallion and good management practices. These mares are considered subfertile and represent a substantial economic and genetic loss to the horse industry (**Coutinho da Silva, 2008**).

Westermann, Parlevliet, Meertens, Sloet van and Oldruitenborgh-Oosterbaan (2003)reported that the main causes of the enlarged ovary in mares are hematomas, anovulatory follicles, abscesses, and neoplasia

Kimura, Hirano, Takemoto, Nambo, Ishinazaka, Himeno, Mishima, Tsumagari, Yokota (2005) stated that the equine ovary has a very unique structure in terms of its extremely large size, the presence of the ovulation fossa and the inverted location of its cortex and medulla. The application of three-dimensional internal structure microscopy (3D-ISM) to observe the mare ovary revealed that the spatial localization of follicles and corpus luteum was clearly visualized by rotating the reconstructed image of the ovary. The extraction of the images of follicles and corpus luteum was also available and gave a quantifiable understanding of their structure.

Miro (2012) described the mare ovary and mentioned that the ovarian layers are inverted with respect to other species, with the follicles and corpus luteum found inside the ovary; only large follicles protrude from the organ's surface that renders the making of diagnoses via rectal palpation of the ovaries very difficult. Ultrasound imaging provides another avenue of examination. Since its first use in 1980**(McCue, 2007),** the diagnostic potential of ultrasonography has greatly improved. Color Doppler ultrasonography can even asses blood flow, allowing for much better monitoring of ovarian activity. He used ultrasound, and color Doppler ultrasonography for monitoring mare ovarian function and disease.