Alexandria Journal of Veterinary Sciences
www.alexjvs.com

AJVS. Vol. 55 (2): 154-161. Oct. 2017
DOI: 10.5455/ajvs.284796

Preovulatory Dominant Follicular Size and Serum Nitric Oxide Can Predicate the Conception Rate after Foaling Heat in Straight Egyptian Arabian Mares

Mohammed A. Elmetwally[1]*, Mohamed Ali M. El-Adl[2], Basma H. Marghani[3], Hossam E. Ali[1], Wael B. El-Domany[1], Samy M. Zaabel[1]

[1]Department of Theriogenology, [2]Department of Biochemistry and Chemistry of nutrition, [3]Department of Physiology, Veterinary Medicine Faculty, Mansoura University, 35516 Mansoura Egypt

Key words:
Arabian horse, dominant follicle, nitric oxide, alkaline phosphatase

Correspondence to:
mmetwally@mans.edu.eg

ABSTRACT

Postpartum heat is very important for ability of mare's to conceive. The present study aimed to evaluate ovarian changes and its relation to oxidative stress during early postpartum period in Arabian horses. Further, the predication of conception according to the relationship between sonographic and biochemical changes analyses. Ultrasound imaging of follicular changes and oxidative analyses changes were investigated at day 7-9 postpartum whereas Receiver Operating Characteristic (ROC) curves were used to determine the ability of oxidative stress and ultrasound imaging to predict the conception rate. ROC analyses indicated that nitric oxide predicate the conception rate with sensitivity (79% P<0.003) and dominant follicle size (80-100% P<0.0001). Alkaline phosphatase, catalase, glutathione, glutathione S transferase and total antioxidant capacity (P>0.05) between pregnant and non-pregnant mares. In conclusion, nitric oxide and dominant follicle diameter plays a crucial role in enhancement the conception rate during foaling heat.

1. INTRODUCTION

In mares, seasonal reproductive activity was recorded from spring till late autumn. There is a 3 weeks cycle during their reproductive periods (Rossdale, 1992). Ultrasonography and manual trans-rectal palpation were routinely performed to determine the follicle on the ovary in the heat and estrus phase in mares (Palmer and Driancourt, 1980). Ginther et al., (2005) reported that mare and women have been showed an amazing similarity regards to follicular dynamics and hormonal changes during the inter-ovulatory interval. In addition, Gastal et al., (2006) showed a significant similarity between mare and women in ultrasonographic changes of the preovulatory follicle before ovulation. Follicular size in mares increases in diameter within seven days at an average rate of 2, 7 mm per day (Ginther, 1983). In contrast, England (1996) reported that this increase in follicular size stops 24-48h before ovulation. During the preovulatory period, maximum of the follicles showed a noticeable change in shape from a spherical to a pear-shaped structure, while the reminder follicles retaining their spherical shape (Ginther, 1979).

In mare, estrous period usually takes 4-7 days, and frequently culminates approximately 24 hours after ovulation (England, 1996). Ovulation is a multifaceted sequence of endocrine, biochemical and cytological changes that ends in the collapse of the preovulatory follicle and the ejection of the oocyte from these follicles (Pierson, 1993). Ginther (2000) detected the mean diameter of the preovulatory follicle on day-1 of estrous and found that their diameter ranged from 35 to 58 mm. Diameter of the preovulatory dominant follicle in mares were significantly larger (45 mm) in contrast to, the preovulatory follicle in heifers were significantly smaller (16 mm) (Ginther et al., 2004).

Oxidant system was recorded in numerous reproductive processes including follicular development, ovarian steroid genesis, ovulation, corpus luteum formation, luteolysis, persistance of pregnancy and starting of parturition (Rizzo et al., 2012). Reactive oxygen species (ROS) were increased in mare serum during estrus and it was recommended that their source was through steroidogenesis occurring in granulosa cells (Castillo et al., 2003). Sugino, (2005) reported that ROS at the physiological levels regulate different signal pathways in folliculogenesis, oocyte maturation, ovulation and provide important signals for the initiation of atresia in antral follicles, and apoptosis of their granulosa cells (Tsai-Turton and Luderer, 2006). Excessive production of ROS causes oxidative stress (Das et al., 2006). It was known that oxidative stress initiate pathological processs which affecting female reproduction (Agarwal et al., 2006). Moreover, ROS can damage various vital molecules and structures in oocytes and granulosa cells within the ovarian follicles, and hasten oocyte aging (Goud et al., 2008).

The lipid peroxidation marker in blood and tissues is Malondialdehyde (MDA) levels (Yousef et al., 2009). One of the most important antioxidant enzymes in biological system is Superoxide dismutase (SOD), which converts superoxide anion into hydrogen peroxide which in turn transformed into water under the effect of catalase and glutathione peroxidase (Rahman et al., 2006). Among to the most dangerous radicals, the superoxide radical that when reacted with NO produces peroxynitrite, a strong ROS, if not removed by endogenous antioxidants enzymes Superoxide dismutase (SOD) and Reduced glutathione GSH), will result in oxidative stress as the rate of oxidation will exceed the rate of anti-oxidation (Grieve and Shah, 2003). Nitric oxide (NO) is synthesized from amino acid L-arginine in the presence of nitric oxide synthase, which exert a vital role during pregnancy in the process of angiogenesis through its vasodilator effect (Li et al., 2009; Wu, 2009) and polyamine synthesis (Lenis et al. 2017). NO stimulates gonadotropin-releasing hormone (GnRH) secretion (Bonello et al., 1996). It regulates gonadotrophin secretion at both hypothalamic and hypophyseal levels (Dixit and Parvizi, 2001). Estrogen may mediate its vascular effects through stimulation of endothelial NO synthesis (Mendelsohn and Karas, 1994). It controls ovarian blood flow before ovulation (Mitsube et al., 2002). In mares, NO seems to be implicated in both in follicular growth and ovulation (Pinto et al., 2002) and is also a potential mediator of luteal development and maintenance, angiogenesis, and blood flow (Ferreira et al., 2011). Also, Honnens et al., (2011) found that NO regulates uterine blood flow during the estrous cycle in mares. Therefore, the present study aimed to evaluate ovarian changes and its relation to oxidative stress during early postpartum period in Arabian horses. Further, the predication of conception according to the relationship between sonographic and biochemical changes analyses.

2. MATERIALS AND METHODS

2.1. Mare management

This study was performed between September 2015 and May 2016 (winter season) using 33 sound straight Egyptian mares between 5 to 9 years in Al Amin horse stud located at Route No. 6, Ahmed Oraby, Society, Cairo - Ismailia Road, Egypt. Rectal palpation and ultrasonographic imaging were thoroughly used for examination of experimental mares using Chison® ultrasound machine (CHISON 600M PORTABLE ULTRASOUND, China). The mares which used in the present study were physiologically normal. All experimental procedures were approved by the Animal Care Committee of Mansoura University.

All foaling dates were known and ovarian and uterine changes were investigated daily between days 4 and 12 postpartum. The acceptance of the mares to the stallion was examined before each examination. Furthermore, uterine and ovarian statuses were determined via rectal palpation as well as ultrasound imaging. The follicular numbers and diameters were determined on both ovaries.

2.2. Blood sampling

Blood samples were collected from all experimental mares via Jugular venipuncture in plain vacuum tubes before either rectal palpation or ultrasound imaging prior to each examination. The harvested sera were obtained after centrifugation of blood samples at (3000 g/15 minutes) and stored at -20°C till biochemical analysis.

2.3. Biochemical analysis

Sera were used for estimation of alkaline phosphatase (ALP, Read and Babson, 1960), malondialdehyde (Cat number MD2120, MDA, Draper and Hadley, 1990), catalase (Cat number SD2521, CAT, Aebi, 1984), glutathione (GSH, Beutler et al., 1963), nitric oxide (Cat number NO2533, NO, Montgomery and Dymock, 1961), glutamine S transferase (Cat number GR2511, GST, Habig et al., 1976),) and total antioxidant capacity (TAC, Koracevic et al., 2001)) using (Bio diagnostic, Egypt) supplied kits (Kit number MD2529).

2.4. Statistical analysis

Normality of quantitative parameters (biochemical biomarkers, diameter and numbers of follicles, number of services per conception) was assessed using normal probability plots and the Kolmogorov-Smirnov test generated with the UNIVARIATE procedure of SAS. All experimental data are expressed as mean ± SEM (Standard error). Follicular number, diameter; numbers of services per conception were compared using a t test using SAS. Receiver Operating Characteristic (ROC) using SPSS (IBM version 20, USA) Statistical analyses were done using SAS® (version 9.2, SAS Institute, Cary, NC, USA). Differences were considered to be significant at ($P \leq 0.05$).

3. RESULTS

The cross talk between mares and stallion (Elmetwally scoring) according to the size of follicular diameter by ultrasound imaging of dominant follicles diameters and prior to collection of blood samples was categorized into: a. Score 1: Adores mares that playing, sniffing and stand to be mated by the stallion (dominant follicle 40± 3.22-46± 1.03 mm) b. Score 2: mares enjoy conversation with stallion but, not accept his advances (dominant follicle ≥30±2.45 ≤ 40±1.78mm) c. Score 3: Resistant mares for any stallion advances (dominant follicle ≥20± 4.57 ≤ 30± 2.84mm, Fig. 1). Ultrasound imaging at day 33 post service indicated a pregnancy rate 66.66 % (22/33) while 33.33 % (11/33) were open mares.

3.1. Follicular dynamic changes during puerperium in straight Egyptian mares

The number of follicles per each ovary and dominant follicle diameters were investigated in this study. The mean total follicular numbers in left ovary was increased by right than left ovaries (Fig. 2b, P>0.05). The mean diameters of dominant follicles in left and right ovaries were (40.27±2.11& 22.68±2.7) in pregnant and nonpregnant mares respectively (Fig. 2a). The number of services per conception was showed tendency to increase in non-pregnant than pregnant mares (P=0.08) and correlated to increased number of follicles at left than right ovaries (R=0.4, P<0.03) (Table.1). Further, the conception rate was tendencies enhanced during mating after day 7 postpartum (P=0.08). Receiver Operating Characteristic (ROC) for ultrasound imaging parameters indicated that the diameter of dominant follicle can predicate pregnancy by 90 % while total number of follicles had 40 % predicative value (Fig. 4b) than total follicular numbers (Fig. 4c).

Table 1: Spearmen's Correlation Coefficient between ultrasound and biochemical parameters

parameters	ALP	MDA	CAT	GSH	NO	GST	TAC	DF	FN	N_C	ROV	LOV
ALP	1	.208	.128	-.355-*	.043	-.064-	-.114-	-.231-	.262	.180	.089	.054
		.217	**.452**	**.031**	**.801**	**.707**	**.503**	**.176**	**.123**	**.295**	**.617**	**.763**
MDA	.208	1	.177	-.215-	.166	.067	-.096-	.135	.208	.194	.062	.099
	.217		**.295**	**.202**	**.325**	**.696**	**.573**	**.432**	**.224**	**.256**	**.729**	**.577**
CAT	.128	.177	1	.097	.206	-.065-	.259	-.174-	.159	.293	.005	-.259-
	.452	**.295**		**.567**	**.222**	**.702**	**.121**	**.311**	**.355**	**.083**	**.979**	**.139**
GSH	-.355-*	-.215-	.097	1	.056	.037	.008	.011	.023	-.243-	.226	-.058-
	.031	**.202**	**.567**		**.741**	**.829**	**.961**	**.951**	**.896**	**.153**	**.200**	**.744**
NO	.043	.166	.206	.056	1	.209	-.123-	.384*	-.188-	.075	-.364-*	-.110-
	.801	**.325**	**.222**	**.741**		**.214**	**.467**	**.021**	**.272**	**.665**	**.035**	**.535**
GST	-.064-	.067	-.065-	.037	.209	1	-.097-	.164	-.130-	-.069-	-.206-	-.046-
	.707	**.696**	**.702**	**.829**	**.214**		**.569**	**.338**	**.449**	**.691**	**.242**	**.796**
TAC	-.114-	-.096-	.259	.008	-.123-	-.097-	1	-.221-	.227	.136	.186	.135
	.503	**.573**	**.121**	**.961**	**.467**	**.569**		**.196**	**.183**	**.431**	**.292**	**.446**
DF	-.231-	.135	-.174-	.011	.384*	.164	-.221-	1	0.212	-.050-	-.277-	-.155-
	.176	**.432**	**.311**	**.951**	**.021**	**.338**	**.196**		**.265**	**.774**	**.113**	**.381**
FN	.262	.208	.159	.023	-.188-	-.130-	.227	0.212	1	.648**	.784**	.119
	.123	**.224**	**.355**	**.896**	**.272**	**.449**	**.183**	**.265**		**.000**	**.000**	**.503**
N_C	.054	.099	-.259-	-.058-	-.110-	-.046-	.135	.119	-.155-	1	.383*	-.121-
	.763	**.577**	**.139**	**.744**	**.535**	**.796**	**.446**	**.503**	**.381**		**.031**	**.497**

Abbreviations: ALP, alkaline phosphatase; MDA, malondialdehyde; CAT, catalase, GSH, reduced glutathione; NO, nitric oxide; GST, glutathione S transferase; TAC, total antioxidant capacity; DF, Dominant follicle; FN, follicle numbers; N_C, number of services per conception; bold means p values; reverse bold means R values

Figure 1: acceptancebehavior between mare and stallion, A: Adores; B: Enjoy his conversation but not his advances and C: Resistant mare

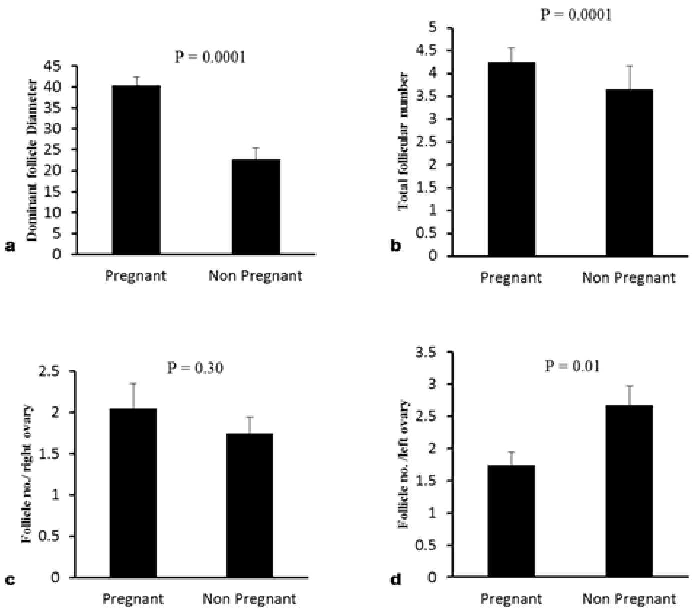

Figure 2: Ultrasound imaging measurements; a: Dominant follicle diameter (mm); b: total follicular number (for left and right ovaries); c: follicle numbers by right ovary and d: follicle number by left ovary. Significant effects are indicated by star ($P < 0.05$). All quantitative data are presented as means± SEM.

3.2. Oxidative stress biomarkers and follicular changes

The correlation coefficient between alkaline phosphatase biomarker (ALP), oxidative stress biomarkers (MDA, GSH, SOD, TAC, and NO) and ultrasound imaging of the ovaries in mares were investigated using ROC curve. We investigated that nitric oxide could predicate pregnancy by about 80 % (Fig. 4a, $P<0.003$) and significantly increased than other biochemical markers (Fig. 3, $P<0.05$). Furthermore, Spearman's Rank Correlation Coefficient for nitric oxide with ultrasound imaging parameters indicated a positive correlation with diameter of dominant follicle ($R=0.4$, $P<0.02$) and negatively correlated with increased follicular number by left than right ovaries ($R=-0.3$, $P<0.04$) (Table.1).

3. DISCUSSION

Breeding of mares at the foal heat is preferable management while anestrus and low pregnancy rate commonly noticed when mares not bred at first foaling heat (Tolksdorff et al., 1976). Furthermore, predicting the day of ovulation should be considered in order to have a higher conception rate after mating and/or insemination of mares at first postpartum heat (Ginther, 2000). Breeding after ovulation decreases the possibility of a successful pregnancy (koskinen, et al., 1990). Equine breeders use symptomatic signs to determine the time of ovulation; anxiety, the complexity of the ovulation mechanisms and the duration of estrous, estrous signs and ovulation time variability are responsible that none of these parameters brings forward evidence with security that the ovulation is close (Roig et al., 2004).

We investigated for the first time in Arabian horses the relation between behavioral acceptances of mares to be standing for mating by stallion during early postpartum period, where we scoring these mares into 3 scores a. Score 1: adores mares that playing, sniffing and stand to be mated by the stallion (dominant follicle 40± 3.22-46± 1.03 mm) b. Score 2: mares enjoy conversation with stallion but not his advances (dominant follicle ≥30 ≤ 40mm) c. Score 3: resistant mares for any stallion advances (dominant follicle ≥20± 4.57 ≤ 30± 2.84mm). Previous studies illustrated the potential role for ultrasound imaging of ovarian changes in equine reproduction (Pierson and Ginther, 1985, Ginther, 2000, Roig et al., 2004). Roig et al., (2004) categories the mares according to exhibition of estrus behavior and dominant follicular size into (0 points - <35mm, 1 point - 35-39mm, 2 points - 40 – 44mm, 3 points - >45mm). The behavioral signs of approaching estrus during first postpartum heat in the present study were similar to the previous reports showed that behavioral signs are occurring most frequently during estrous such as restlessness, tail raising, calm urination and posturing (Back et al., 1974; Squires, 1993; England, 1996).

In the present study we investigated the importance of oxidative stress biomarkers during early postpartum in Arabian mares during winter season. Furthermore, the present data indicates that Arabian horses did not affected by seasonality like other horse breeds. From all biochemical biomarkers malondialdehyde and nitric oxide were significantly higher in pregnant than nonpregnant mares during early puerperium. Similar observation for increased MDA in pregnancy was reported by Patil et al., (2004) we found significant increase in lipid peroxides in all the three trimesters of pregnancy as compared to levels in nonpregnant women. Moreover Xiao et al., (2006) found that lipid peroxidation increased in pregnant rabbits and still increased as gestational age advanced during pregnancy. Mueller et al., (2005) suggested that placenta is the major source of pro-oxidant agents and antioxidant enzyme systems. As the development of placenta, the production of lipid peroxidation increased gradually. Other studies illustrated the lower level of Malondialdehyde (product of lipid peroxidation) with advancement of pregnancy than peri-implantation stage (Atiba et al., 2014). Nitric oxide is a potent vasodilator and increased blood flow to different body tissue (Bode-Bogeret et al., 2007). Moreover, Nitric oxide appears, under normal physiologic conditions, to be the most important regulator of blood flow (Klubunde, 2015). Welter et al., (2004) reported that different isoforms of NO synthase were present in the equine endometrium of early pregnant mares. NO is considered a vital mediator concerned in maintenance of pregnancy (Yalampalli et al., 1994). The increased pregnancy rate in response to increased nitric oxide may be attributed its effects of follicular blood flow and subsequent improvement of ovulated oocyte and its potential role in enhancement of luteal blood flow and maintenance of luteal function during early pregnancy (Li et al., 2009). Similarly, Silva et al., (2006) reported that pregnancy rates were enhanced in response to increased follicular blood flow in mare.

In the present study, there was no significant changes of alkaline phosphatase, catalase, glutathione, total antioxidant capacity, glutamine S transferase, total antioxidant capacity (P>0.05) between pregnant and non pregnant mares. These results were in agreement with previous results in human being (Yüksel and Yiğit, 2015) which indicated that superoxide dismutase glutathione peroxidase and catalase were not changed during first trimester in pregnant than non-pregnant women. Meanwhile, these parameters were decreased significantly in third trimester when compared with early pregnancy.

We investigated in the present study the importance of both nitric oxide and dominant follicle diameter as crucial biomarkers to predicate the incidence of pregnancy and enhanced conception rate rather than, the follicle numbers and other biochemical changes. This finding indicated the importance between the effects of nitric oxide on follicular blood flow and ovulated oocyte quality. These results were similar to that in pigs and sheep (lie et al., 2009; Kwon et al., 2004).

Altogether, results of the present study indicated the importance of dominant follicle diameter and serum nitric oxide concentration at time of mating during early puerperium on conception rate in Arabian horses. Further, we validated the relationship between behavioral changes (Elmetwally scoring), dominant follicles diameters and conception rate during foaling heat. Moreover, the present data illustrated absence of seasonality in straight Egyptian Arabian mares where all breeders prefer to breed their mares during winter (September to February) than summer season.

5. CONCLUSION

The ultrasound imaging and blood analyses during early postpartum constitutes a cruciate role in next conception rate in Arabian horses. Furthermore, regular estrous cycle between September and May indicated the absence of seasonality in Arabian horses wherever most of Egyptian breeders prefer to breed their mares during winter than summer season. Altogether, serum nitric oxide and dominant follicle size are potential factors that predicates conception rate during physiological condition than the preovulatory oxidative stress changes.

6. ACKNOWLEDGEMENTS

We want to acknowledge Mr Mohammed Al-Amin and all workers at Al-Amin Horse Stud for helping us during the period of this study.

7. REFERENCES

Aebi, H. 1984. Catalase in vitro. Methods in enzymology. 105:121-126.

Agarwal, A., S. Gupta, S. Sikka. 2006. The role of free radicals and antioxidants in reproduction. Current Opinion in Obst. and Gyn. 18:325.

Atiba, A. S., F. M. Abbiyesuku, D. A. Adekanle, D. P. Oparinde, O. A. Ajose, T. A. Niran-Atiba. 2014. Malondialdehyde and antioxidant enzymes in second and third trimesters of pre-eclamptic Nigerian women. Niger Postgrad Med. J. 21(2):150-154.

Bode-Böger, S. M., F. Scalera, L. J. Ignarro 2007. The L-arginne paradox: the importance of the L-arginine/asymmetrical dimethylarginine ratio. Phamacol. Therap. 114:295-306.

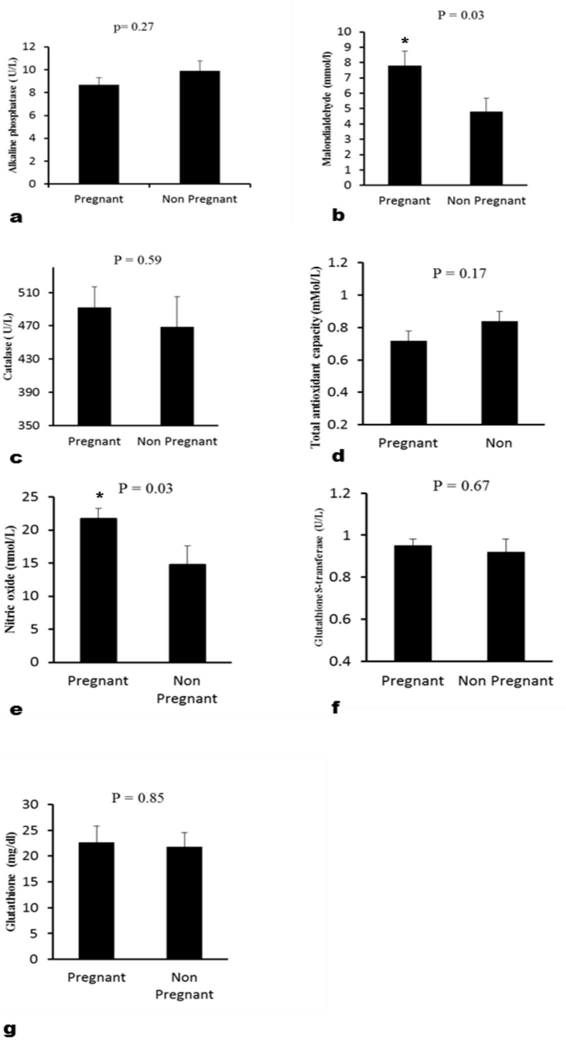

Figure 3: oxidative stress biomarkers in pregnant vs non pregnant mares. A: Alkaline phosphatase (U/L); b: Malondialdehyde (mmol/l); c: Catalase (U/L); d: Glutathione (mg/dl); e: Nitric oxide (nmol/L); f: Glutathione S-transferase (U/L) and g: Total antioxidant capacity (mMol/L). Significant effects are indicated by star (P < 0.05). All quantitative data are presented as means± SEM.

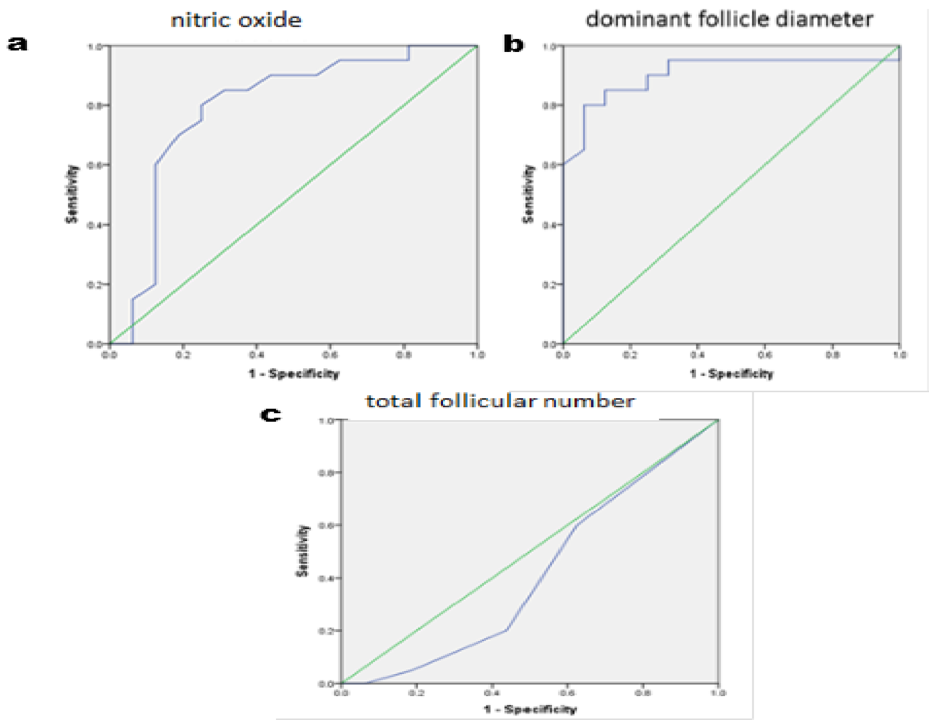

Figure 4: Receiver operating characteristic curve (ROC) show sensitivity levels to predicate pregnancy via serum nitric oxide (a), dominant follicle diameter (b) and total follicular number (c)

Bonello, N., K. Mckie, M. Jasper, L. Andrew, N. Ross, E. Brayborn. 1996. Inhibition of nitric oxide: effects of interleukin-16 enhanced ovulation rate, steroid hormones, and ovarian leukocytes distribution at ovulation in rat. Biol. Reprod. 54(2): 436-445.

Castillo, C., J. Hernández, M. López-Alonso, M. Miranda, J. L. Benedito. 2003. Values of plasma lipid hydroperoxides and total antioxidant status in healthy dairy cows: Preliminary observations. Arch. Tierz. 46(3): 227-233.

Das, S., R. Chattopadhyay, S. Ghosh, S. K. Goswami, B. N. Chakravarty. 2006. Reactive oxygen species level in follicular fluid–embryo quality marker in IVF. Hum. Reprod. 21: 2403–2407.

Dixit, V. D., N. Parvizi. 2001. Nitric oxide and the control of reproduction. Anim. Reprod. Sci. 65(1-2):1-16.

Draper, H., M. Hadley. 1990. Malondialdehyde determination as index of lipid Peroxidation. Methods in enzymology. 186:421-431.

England, G. 1996. Allen's Fertility and Obstetrics in the Horse. 2nd ed. Blackwell Sci. Oxford, London. Pages 241.

Ferreira-Dias, G., A. S. Gosta, L. Mateus, A. J. Korzekwa, A. Galvao, A. D. Redmer. 2011. Nitric oxide stimulates progesterone and prostaglandin E secretion as well as argiogenic activity in the equine corpus luteum. Dom. Anim. Endocrinol. 40(1):1-9.

Gastal, E. L., M. O. Gastal, O. J. Ginther. 2006. Relationships of changes in B-mode echotexture and colour-Doppler signals in the wall of the preovulatory follicle to changes in systemic oestradiol concentrations and the effects of human chorionic gonadotrophin in mares. Reprod. 131(4):699-709.

Ginther, O. J. 1979. Reproductive Biology of the Mare. Basic and applied aspects. Equiservices, Cross Plains, Wisconsin, USA. Pages 133-154.

Ginther, O. J. 1983. Sexual behaviour following introduction of stallion into a group of mares. Theriogenol. 19:877-886.

Ginther, O. J. 2000. Selection of the dominant follicle in cattle and horses. Anim. Reprod. Sci. 60:61-79.

Ginther, O. J., M. A. Beg, E. L. Gastal, M. O. Gastal, A. R. Baerwald, R. A. Pierson. 2005. Systemic concentrations of hormones during the development of follicular waves in mares and women: a comparative study. Reprod. 130(3):379-388.

Ginther, O. J., D. R. Bergfelt, M. A. Beg, C. Meira, K. Kot. 2004. In vivo effects of an intrafollicular injection of insulin-like growth factor 1 on the mechanism of follicle deviation in heifers and mares. Bio. of Reprod. (70):99-105.

Goud, A. P., P. T. Goud, M. P. Diamond, B. Gonik, H. M. Abu-Soud. 2008. Reactive oxygen species and oocyte aging: role of superoxide, hydrogen peroxide, and hypochlorous acid. Free Radical Bio. and Med. 44:1295–1304.

Grieve, D. J., A. M. Shah. 2003. Oxidative stress in heart failure. More than just damage. Eur. Heart. J. 24: 2161–2163.

Habig, W. H., M. J. Pabst, W. B. Jakoby. 1976. Glutathione S-transferase AA from rat liver. Arch. Bioch. and Bioph. 175: 710-716.

Honnens, A., S. Weisser, H. Welter, R. Einspanier, H. Bollwein. 2011. Relationship between uterine blood flow, peripheral sex steroids, expression of endometrial estrogen receptors and nitric oxide synthases during the estrous cycle in mares. J. Reprod. Develop. 57(1):43-48.

Klunbude, R. E. 2015. Cardiovascular Physiology Concepts, second ed. Lippincott Williams & Wilkins. Baltimore, MD, USA. Turk. J. Med. Sci. 45(2):454-459.

Koracevic, D., G. Koracevic, V. Djordjevic, S. Andrejevic, V. Cosic. 2001. Method for the measurement of antioxidant activity in human fluids. J. clin. Path. 54:356-361.

Koskinen, E., H. linderberg, H. Kuntsi, I. Ruotsalainen, T. Katila. 1990. Fertility of mares after postovulatory insemination. J. Vet. Med. 37:77-80.

Kwon, H., G. Wu, C. J. Meininger, F. W. Bazer, T. E. Spencer. 2004. Developmental changes in nitric oxide synthesis in the ovine placenta. Biol. Reprod. 70(3):679-686.

Lenis, Y. Y., M. A. Elmetwally, J. G. Maldonado-Estrada, F. W. Bazer. 2017. Physiological importance of polyamines. Zygote. 25(3):244-255.

Li, X., F. W. Bazer, H. Gao, W. Jobgen, G. A. Johnson, P. Li, J. R. McKnight, M. C. Satterfield, T. E. Spencer, G. Wu. 2009. Amino acids and gaseous signaling. Amino Acids. 37:65–78.

Mendelsohn, M .E., R. H. Karas. 1994. Estrogen and the blood vessel wall. Curr. opinion cardiol. 9(5):619-626.

Mitsube, K., U. Zackrisson, M. Brannstrom. 2002. Nitric oxide regulates ovarian blood flow in the rat durin the periovulatory period. Hum. Reprod. 17(10):2509-2516.

Montgomery, H., J. F. Dymock. 1961. Determination of nitrite in water (Ryal Soc Chemistry Thomas Graham House, Sci. Park, Milton RD, Cambridge CB4 WF, Cambs, England. 414.

Mueller, A., C. Koebnick, H. Binder. 2005. Placental defense is considered sufficient to control lipid peroxidation in pregnancy. Med. Hypotheses. 64:553-557.

Palmer, E., M. A. Driancourt. 1980. Use of ultrasound echography in equine gynecology. Theriogenol. 13:203-216.

Patil, S. B., M. V. Kodliwadmath, M. Sheela, M. Kodliwadmath. 2006. Lipid peroxidation and nonenzymatic antioxidants in normal pregnancy. J. Obstet. Gynecol. 56(5):399-401.

Pierson, R. A. 1993. Folliculogenesis and Ovulation. In: Equine Reproduction. Angus, O., J. McKinnon, J.L. Voss, (Eds). Lea & Febiger. Philadelphia/London. Pages161-171.

Pierson, R. A., O. J. Ginther. 1985. Ultrasonic evaluation of the preovulatory follicle in the mare. Theriogenol. 24:359-368.

Pinto, C. R. F., D. L. Paccamonti, B. E. Eilts, C. R. Short, R. A. Godke. 2002. Effect of nitric oxide synthase inhibitors on ovulation in HCG stimulates mares, Theriogenol. 58(5):1017-1026.

Rahman, I., S. K. Biswas, A. Kode. 2006. Oxidant and antioxidant balance in the airways and airway diseases. Eur. J. Pharmacol. 533: 222–39.

Read, P. A., A. L. Babson. 1960. Rapid method for the quantitative determination of serum alkaline phosphatase. Clin. Chem. 6(3): 269-275.

Rizzo, A., M. T. Roscino, F. Binetti, R. L. FSciorsci. 2012. Roles of reactive oxygen species in female reproduction. Reprod. Domest. Anim. 47(2):344-52.

Roig, J. M., F. Muñoz, J. Piedrafita, Y. A. Quintero-Moreno. 2004. Prediction of the day of ovulation in mares through physiological parameters measured during estrous. Revista Científica. 11(1):54-58.

Rossdale, P. D. 1992. Horse breeding, David &Charles, Newton Abot, Devon, Anatomy and physiology of reproduction in the mare. P. 351-380.

Silva, L. A., E. L. Gastal, M. O. Gastal, M. A. Beg, J. Ginther. 2006. Relationship between vascularity of the preovulatory follicle and establishment of pregnancy in mares. Anim. Reprod. 3:339–346.

Squires, E. L. 1993. Estrous detection. In: Equine Reproduction. Angus, O.; McKinnon, J. Voss. J. L. (Eds). Lea & Febiger. Philadelphia/London. Pages 186-195.

Sugino, N. 2005. Reactive oxygen species in ovarian physiology. Reprod. Med. Bio. 4: 31–44.

Tolksdorff, E., W. Jöchle, D. R. Lamond, E. Klug, H. Merkt. 1976. Induction of ovulation during the postpartum period in the thoroughbred mare with a prostaglandin analogue, Synchrocept. Theriogenol. 6(4):403–412.

Tsai-Turton, M., U. Luderer. 2006. Opposing effects of glutathione depletion and follicle-stimulating hormone on reactive oxygen species and apoptosis in cultured preovulatory rat follicles. Endocrinol. 147:1224.

Welter, H., H. Bollwein, F. Weber, S. Rohn, R. Einspanier. 2004. Expression of endothelial and inducible nitric oxide synthases is modulated in the endometrium of cyclic and early pregnant mares. Reprod. Fertil. Dev. 16(7): 689–698.

Wu, G. 2009. Amino acids: metabolism, functions, and nutrition. Amino Acids. 37:1–17.

Yallampalli, C., H. Izumi, M. Byam-Smith, R. E. Garfield. 1994. L arginine: nitric oxide-GMP system exists in the uterus and inhibits contractility during pregnancy. American Journal of Obstetrics and Gynecology. 170:175–185.

Yousef, M. I., A. A. Saad, L. K. El-Shennawy. 2009. Protective effect of grape seed proanthocyanidin extract against oxidative stress induced by cisplatin in rats. Food Chem. Toxicol. 46:1176–83.

Yüksel, S., A. A. Yiğit. 2015. Malondialdehyde and nitric oxide levels and catalase, superoxide dismutase, and glutathione peroxidase levels in maternal blood during different trimesters of pregnancy and in the cord blood of newborns.