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Correlation between the serum and follicular fluid vascular endothelial growth factor A and nitric oxide levels and follicular vascularity in Arabian mares

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1. Introduction

The use of color Doppler ultrasound is an emerging technique that allows non-invasive evaluation of the blood flow of various organs. In the mare, the blood flow of the uterine and ovarian arteries, follicle wall, corpus luteum, endometrium, placenta, umbilical cord and various embryonic and fetal structures was studied using color Doppler ultrasound evaluation [1]. Vascular endothelial growth factor (VEGF) and nitric oxide (NO) have been known to be potent vasodilator and angiogenic factors and play an important role in ovarian angiogenesis during folliculogenesis and ovulation. NO mediates the vascular permeability of VEGF while VEGF stimulates the production of NO in endothelial cells [2]. In this study, we examined the following hypotheses: (1) follicular blood flow is altered in older mares when compared to young mares; (2) follicle blood flow is affected by hCG treatment. The objectives of this experiment were to determine the effects of the mare's age and hCG treatments on dominant follicle vascularity and to establish the correlation between the concentration of VEGFA and NO in blood and in follicular fluid.

2. Materials and Methods

The study was carried out from February through June in the Northern Hemisphere with the inclusion of 27 non-lactating Arabian mares. Fifteen of the mares were younger (3-10 y) and 12 were older (19-23 y). Reproductive tracts were examined using the transrectal B-mode ultrasonographic device (Esaote Pie Medikal MyLab Five Vet) equipped with a 7.5 MHz linear transducer. Multiple images were saved as .bmp format files. A region of interest that contains the follicle wall was determined and analyzed using ImageJ public domain software (National Institutes of Health; http://rsb.info.nih.gov/ij/). Quantification analyses were performed over the segmented areas for the calculation of areal dimensions presenting follicular vascularity based on pixels. When a follicle $\geq$35 mm was imaged, follicular blood flow (FBF) was measured (Day-1) with color doppler ultrasonography. Jugular blood was collected at the time of ultrasonography. After that hCG (2500 IU, I.V. Chorulon, MSD, Turkey) was administered to 14 mares who served as the hCG-treated group (hCG +). hCG was not administered to the remaining 13 mares who served as the non- hCG treated group (hCG -). 24 hour later (just prior to follicle aspiration) follicular blood flow (FBF) was measured (Day 0) and jugular blood was collected again. Concentrations of VEGFA and NO in follicular fluid and serum samples were assayed with the ELISA test kit (Qayee-Bio, China). According to the manufacturer, the plate coefficient of variation was less than 15%, the intra and inter assay reliability was high while their coefficients were low. Two-way Anova procedures were used to analyze follicular blood flow, VEGFA and NO. Correlation between the concentrations of VEGFA and NO in blood and in follicular fluid was calculated by Pearson correlation analysis.

3. Results

Age affected follicle vascularity, as measured (Figure 1) by the percentage of color pixels per total pixels in the follicle wall. Vascularity of follicles was not different for old and young mares in day -1 and day 0 (Table 1). Vascularity of follicles was not affected by treatment with hCG. Blood VEGFA concentration for day 0 in the hCG treated group was lower than the non-treated group ($P<.05$) (Table 2). There was no difference for follicular fluid VEGFA

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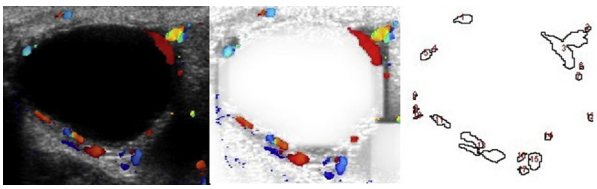


Fig. 1. Analysis of doppler image

concentration between groups treated and not treated with hCG and the same was also applicable for young and old mares. Blood NO concentration for day -1 and day 0 was not affected by treatment with hCG and age. There was no difference for follicular fluid NO concentration between groups treated and non-treated with hCG and the same was also applicable for young and old mares (Table 2). A positive correlation ($r=0.73$) ($p<0.05$) was observed between blood

Table 1
The comparison of Follicular Blood Flow (pixel) Follicular Blood Flow Day -1 (FBF-1), Follicular Blood Flow Day 0 (FBF-0), hCG treated (hCG+), untreated (hCG-), young (3-10 y), old (19-23 y)

	Treatment		Age	
	hCG+	hCG-	Young	Old
FBF -1	926 ± 83	911 ± 86	837 ± 80	1001 ± 417
FBF 0	1242 ± 134	1328 ± 138	1317 ± 128	1253 ± 143

Table 2
The comparison of VEGFA and NO concentrations in follicular fluid and blood in young and old mares and the hCG treated and non-treated groups. Blood Vascular Endothelial Growth Factor A Day -1 (BVEGF -1), Blood Vascular Endothelial Growth Factor A Day 0 (BVEGF 0), Follicular Fluid Vascular Endothelial Growth Factor A (VEGF fol), Blood Nitric Oxide Day -1 (BNO -1), Blood Nitric Oxide Day -0 (BNO -0), Follicular Fluid Nitric Oxide (NO fol)

	VEGF			NO		
	BVEGF-1	BVEGF 0	VEGF fol	BNO -1	BNO 0	NO fol
hCG+	732 ± 46	798 ± 30[c]	1169 ± 44	106 ± 6	119 ± 11	129 ± 5
hCG-	880 ± 150	988 ± 31[d]	1213 ± 46	100 ± 7	126 ± 11	128 ± 6
Young	892 ± 44[a]	969 ± 29[e]	1168 ± 43	99 ± 6	118 ± 11	125 ± 5
Old	720 ± 49[b]	817 ± 32[f]	1214 ± 48	107 ± 7	127 ± 12	133 ± 6

[(a b), (e f)] Values with different subscripts in columns were different ($P < 0.05$)
[(c d)] Values with different subscripts in columns were different ($P < 0.001$).

VEGFA level for Day -1 and Day 0 whereas a negative correlation ($r=0.48$) ($p<0.05$) was detected between follicular fluid VEGFA level and follicular fluid NO level.

4. Discussion

In our study, follicular blood flow was not different in young and old mares. In addition hCG treatment was not affected by follicle vascularity. Blood VEGFA concentration for day-1 in young mares was higher than old mares. VEGF and NO are angiogenic factors but we did find not any correlation between blood VEGFA and NO and follicle vascularity. We found negative correlation between follicular fluid VEGFA and follicular fluid NO. Friedman et al. [3] reported that VEGF in the FF of women of advanced reproductive age was elevated compared with that of younger women. They suggested that the increasing VEGF level was caused by a hypoxic environment within the follicles of older women. It has been reported that VEGF did not reveal significant differences in the mature group versus immature oocytes, but VEGF with fertilized oocytes was significantly higher than non-fertilized oocytes in mature oocytes [4]. We did not directly evaluate oocytes but we previously reported [5] that VEGF levels in young mares were not different between cases when mature and intermediate oocytes were collected, whereas the levels in old mares when mature oocytes were collected were higher than cases when intermediate and immature oocytes were collected.

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References

[1] Hendriks-Onstein WK, Stout TAE. The use of color Doppler ultrasound during fertility management of the mare. http://www.uu.nl/faculty/veterinarymedicine/NL/Actueel/media/2010/november/Documents/20101101%20Esaote.pdf Accessed June 30, 2013.
[2] Choong-Sik H, Bo-Sun J, Seung-Chul K, Jong-Kil J, Hwi-Gon K, Kyu-Sup L. Estrogen administration during superovulation increases oocyte quality and expressions of vascular endothelial growth factor and nitric oxide synthase in the ovary. J Obstet Gynaecol Res Vol 2010;36:789–95.
[3] Friedman CI, Seifer DB, Kennard EA, Arbogast L, Alak B, Danforth DR. Elevated level of follicular fluid vascular endothelial growth factor is a marker of diminished pregnancy potential. Fertil Steril 1998;70:836–9.
[4] Malamitsi-Puchner A, Sarandakau A, Baka SG, Tziotis J, Rizos D, Hassiakos D, Creatsas G. Concentrations of angiogenic factors in follicular fluid and oocyte-cumulus complex culture medium from women undergoing in vitro fertilization: association with oocyte maturity and fertilization. Fertil Steril 2001;76:98–101.
[5] Gunduz MC, Kaşıkcı G, Yılmaz OT, Enginler SO, Ucmak M, Ateş A, Gursel FE, Evkuran G, Tore MD. Can the vascular endothelial growth factor (VEGF) and total estradiol 17β be used as a marker of equine oocyte maturation? Turk J Vet Anim Sci 2011;35:331–5.