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Can the vascular endothelial growth factor (VEGF) and total estradiol 17β be used as a marker of equine oocyte maturation?

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Abstract: The aim of this study was to quantitate vascular endothelial growth factor (VEGF) and estradiol-17β in follicular fluid (FF) and serum and to correlate the levels of these substances with oocyte maturation. Transvaginal ultrasound-guided follicular aspirations were performed with the purpose of oocyte collection when the follicle was >35 mm in diameter. The mares were divided into 2 groups according to age, and the changes in VEGF and estradiol-17β in follicular fluid and serum were examined. Levels of estradiol-17β in the follicular fluid and serum of young mares (4-10 years old) did not differ from those in old mares (20-24 years old) ($P > 0.05$). The follicular fluid and serum concentrations of VEGF in old mares were lower ($P < 0.001$) than those in young mares. The VEGF levels in young mares did not differ in cases when either mature or intermediate oocytes were collected, whereas its levels in old mares were higher ($P < 0.01$) when mature oocytes were collected than when intermediate and immature oocytes were collected. Our data suggest that VEGF may play an important role in follicular growth and development, that VEGF levels appear to be age-dependent, and that VEGF levels might be valuable biochemical markers of oocyte maturation.

Key words: VEGF, oocyte maturation, follicular fluid, mare

Vasküler endotelyal büyüme faktörü (VEBF) ve total östradiol 17β kısrak oosit maturasyonunda bir belirleyici olarak kullanılabilir mi?

Özet: Bu çalışmanın amacı folliküler sıvı (FS) ve serumda vasküler endotelyal büyüme faktörü (VEBF) ve total östradiol 17β'yı belirlemek ve bu maddelerin seviyelerini oosit maturasyonu ile ilişkilendirmektir. Follikül çapı 35 mm'den büyük olduğunda; oosit toplama amacıyla transvajinal ultrason rehberliğinde follikül aspirasyonu gerçekleştirildi. Kısraklar yaşa göre iki gruba ayrıldı ve VEBF ve östradiol 17β değişimleri folliküler sıvı ve serumda incelendi. Folliküler sıvı ve serum östradiol 17β seviyeleri genç (4-10 yaş) ve yaşlı (20-24 yaş) kısrakların arasında farklı bulunmadı ($P > 0,05$). Yaşlı kısrakların folliküler sıvı ve serum VEBF konsantrasyonları genç kısraklardakinden daha düşük bulundu ($P < 0,001$). Genç kısraklardan elde edilen VEFB seviyesi mature ve intermediate oositler arasında fark göstermezken; yaşlı kısraklardan elde edilen VEFB seviyesi mature oositlerde intermediate ve immature oositlere göre daha yüksek bulundu ($P < 0,01$). Yapılan çalışma sonucunda; VEBF'nün folliküler büyüme ve gelişimde önemli bir rol oynayabileceği, VEBF seviyelerinin yaşa bağımlı gözüktüğü ve bu nedenle VEFB seviyeleri oosit maturasyonun değerli biyokimyasal belirleyicisi olabileceği kanısına varıldı.

Anahtar sözcükler: VEBF, oosit maturasyonu, folliküler sıvı, kısrak

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Introduction

Although the process of ovarian follicular development and nuclear maturation of oocytes is governed by conventional endocrine principles such as pituitary gonadotropins and ovarian steroids, recent observations suggest that gonadotropins are only part of a complex system of autocrine and paracrine agents, including growth factors (1).

Growth factors play a regulatory role in ovarian function. Vascular endothelial growth factor (VEGF), a protein thought to play a critical role in the regulation of vascular permeability and angiogenesis during embryogenesis and in pathological tissue growth, is also expressed in the ovaries of numerous species (2-4). VEGF production increases in luteinizing granulosa cells of the ovulatory follicle as well as in the developing corpus luteum in primates, suggesting that this factor is important for vascular changes associated with the transition of the follicle to the corpus luteum (5).

In the ovary, VEGF is produced both by granulosa and thecal cells (6,7). A recent study of macaque granulosa cells suggested that VEGF expression increases in response to gonadotrophins such as LH, FSH, and human chorionic gonadotrophin (HCG) (5).

In women with normal non-stimulated cycles and those undergoing IVF, the local VEGF production in follicular fluid is correlated with the degree of follicular luteinization (8,9). Progesterone also appears to play a role in determining VEGF concentration in follicular fluid (10). A positive correlation has been observed between follicular fluid VEGF concentrations and patient age, especially in patients ≥38 years undergoing IVF (11,12). Recently, it has been reported that elevated VEGF concentrations in follicular fluid might predict poor conception rates after IVF (13).

The aim of this study was to quantitate the VEGF and total estradiol 17β in follicular fluid (FF) and serum, and to correlate the levels of these substances with oocyte maturation and mare age.

Materials and methods

The study was conducted during the breeding season on younger (4-10 years old) and older (20-24 years old) non-lactating Arabian mares. Transvaginal ultrasound-guided follicular aspirations were performed with the purpose of oocyte collection when the follicle was >35 mm in diameter.

Oocytes were immediately identified, rinsed twice in culture medium, and placed in a petri dish containing 2.5 mL of culture medium (Tissue Culture Medium 199 with additions of 10% fetal calf serum, 0.2 mM pyruvate, and 50 μg/mL gentamicin). Oocytes were incubated in air containing 6% CO_2 at 38.5 °C for approximately 16 h.

Oocytes were graded based on morphology (scores from 1, excellent, to 4, poor). Cumulus expansion was graded as immature, intermediate, or mature. Grades and maturity were assigned after culture. Blood and follicular fluid were not used from cycles in which oocytes were not recovered. Critical evaluations of oocytes were difficult, as cumulus cells frequently obstructed direct imaging of the oocyte under a stereomicroscope.

Jugular blood (10 mL) was collected at the time of follicular aspiration. Blood samples and follicular fluid were centrifuged at 3500 × *g* for 15 min and stored at –20 °C until being analyzed for VEGF and estradiol-17β. Concentrations of estradiol in follicular fluid and serum were estimated by radioimmunoassay (RIA) using an active estradiol RIA kit (active Estradiol RIA DSL-43100, Webster, TX, USA). The amount of [125I]-labeled estradiol bound to antibody is inversely proportional to the concentration of unlabeled estradiol present. The separation of free and bound antigen is achieved by decanting or aspirating the antibody-coated tubes. The sensitivity, defined as the lowest detectable level of estradiol that can be distinguished from the 0 pg/mL Estradiol Standard, is 11 pg/mL at the 95% confidence limit. The intra-assay precision was determined from the mean of 12 replicates each and the coefficient of variation was 2.01% when the mean was 241.73 pg/mL. The inter-assay precision was determined from the mean of average duplicates for 24 separate runs and the coefficient of variation was 7.37% when the mean was 247.48 pg/mL. The assay has low cross-reactivity with equilenin (6.1%),

estrone (3.4%), 17β-estradiol-3-glucuronide (1.8%), estrone-β-d-glucuronide (0.3%), 16-ketoestradiol (0.29%), 17α-estradiol (0.26%), estradiol-3-sulfate (0.21%), estriol (0.75%), and equilin (0.84%). The results were measured by gamma counter (MGM Instruments, ISOCOMP I, USA).

Concentrations of VEGF in follicular fluid and serum were assayed with the ELISA test kit for Equine Vascular Endothelial Cell Growth Factor (USCN Life Science Inc. Wuhan, China). The minimum detectable dose of equine VEGF is typically less than 0.04 ng/mL. According to the manufacturer this assay has high sensitivity and excellent specificity for detection of equine VEGF. No significant cross-reactivity or interference was observed.

The least squares procedures were used to analyze for VEGF and total estradiol-17β. The model used in the analysis included fixed effects of age (young or old), sample (follicular fluid or serum) and maturation (mature, intermediate, or immature) and 2-way interactions of these effects.

Results

The mares were divided into 2 groups based on age, and the changes in VEGF in follicular fluid and serum were examined (Table). The follicular fluid and serum concentrations of VEGF in old mares were lower ($P < 0.001$) than those in young mares. Levels of estradiol-17β in the follicular fluid and serum of young mares did not differ from those in old mares ($P > 0.05$). Estradiol-17β level in follicular fluid was higher ($P < 0.001$) than that in the serum of the young and old mares. VEGF level in follicular fluid was lower ($P < 0.05$) than that in the serum of the young and old mares.

Oocyte grades varied ($P < 0.05$) with age, with higher grades representing worse morphology scores (young 4-10 years, 1.9 ± 0.1, n = 14 and old 20-24 years, 2.6 ± 0.2, n = 20).

VEGF level for young mares did not differ between when mature and intermediate oocytes were collected whereas its level for old mares when mature oocytes were collected was higher ($P < 0.01$) than when intermediate and immature oocytes were collected (Figure).

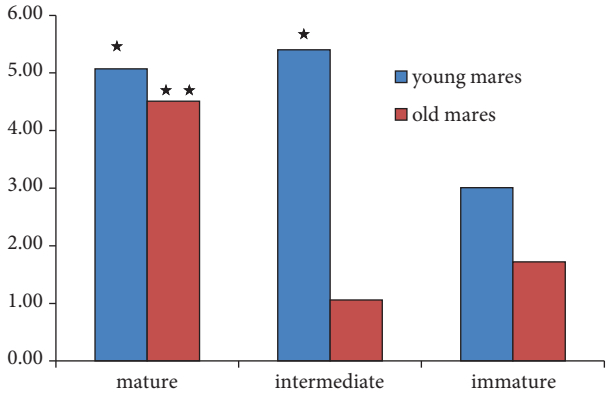

Figure. The mean concentrations of VEGF for young and old mares. Oocytes were classified as immature, intermediate, or mature.

Table. The comparison of VEGF and estradiol-17β concentrations in follicular fluid (FF) and serum in young and old mares.

	VEGF (ng/mL)		Estradiol-17β	
	FF	Serum	FF (pg/mL)	Serum (ng/mL)
Young (n = 14)	3.46 ± 0.47	5.06 ± 0.47	250.7 ± 70.4	68.5 ± 7.2
Old (n = 20)	1.91 ± 0.5	2.9 ± 0.5	303.5 ± 67.3	53.3 ± 4.7
Significance				
Age	$P < 0.001$		$P > 0.05$	
Sample	$P < 0.05$		$P < 0.001$	
Age & Sample	$P > 0.05$		$P > 0.05$	

Discussion

Estradiol-17 β and progesterone have been suggested as markers for oocyte maturity and fertilization in humans (14,15). On the other hand, it has been reported that there is no correlation between oocyte maturity and the concentrations of estradiol-17β and progesterone in FF (16-19). Consequently, no relationship between ovarian steroid hormones and oocyte maturation was observed.

Therefore, we hypothesized that local factors, such as growth factors produced in follicles, might act upon follicular development and oocyte maturation rather than ovarian steroid hormones in mares. In our study the levels of estradiol-17β in the follicular fluid and serum of young mares did not differ from those in old mares. We previously (20) reported that IGF-I and progesterone concentrations were significantly reduced and correlated with oocyte maturation. IGF-I levels in follicular fluid and serum were affected by mare age, and the IGF-I concentrations in follicular fluid from young mares were higher than those from old mares. It was suggested that follicular fluid levels of IGF-I and progesterone could be available biochemical markers of oocyte maturation.

In the present study, we demonstrated that VEGF is present in the follicular fluid. The follicular fluid and serum concentrations of VEGF in old mares were lower than those in young mares. Moreover, VEGF levels in young mares were not different between when mature and intermediate oocytes were collected, whereas its levels in old mares when mature oocytes were collected were higher than when intermediate and immature oocytes were collected. Kawano et al. (21) reported that VEGF was increased in older women. Friedman et al. (13) 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 (22). Our data suggest that VEGF may play an important role in follicular growth and development, that VEGF levels appear to be age-dependent, and that VEGF levels might be valuable biochemical markers of oocyte maturation.

Acknowledgements

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