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VEGF Concentrations Levels in Bovine Ovulatory Follicles after Prostaglandin Treatment

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Abstract: The aim of this study was to evaluate variations in the bovine follicular VEGF concentrations 72 hours after a double prostaglandin synchronisation treatment The experiment was performed on 17 multiparous Italian Friesian dairy cows which were at least 90 days post-calving. The follicular fluid was collected from the dominant follicles under ultrasound guidance. The ratio between the progesterone and estrogen follicular fluid concentrations were used to allow into active and nonactive estrogen follicles. The average VEGF follicular concentrations were significantly different ($P<0.05$) between the two groups. Moreover, the follicular NEFA levels increased significantly ($P<0.01$) in the nonestrogen active group, while its IGF-I concentrations were significantly lower ($P<0.05$). Our data suggest that follicular VEGF and NEFA, as well as IGF-I, may play a key role in bovine follicle growth and could be valuable biochemical markers of oocyte maturation.

Key words: VEGF, Follicle, Bovine, Hormones

Introduction

It is well known that ovarian function is regulated by the cyclical and pulsatory secretion of gonadotropin that leads to ovulation and cyclical steroidogenesis. Several authors have reported no correlation between oocyte maturation and estradiol and progesterone concentrations in the follicular fluid. Local factors produced in the follicles, such as cytochines and/or growth factors can also act on follicle development and oocytes maturation in place of ovarian steroids (Kawano *et al.*, 2003). The evolution and decline of the ovarian structure is linked with angiogenic processes which is a crucial step in physiological processes such as follicle formation and corpus luteum formation. Dysfunctions in ovarian angiogenesis can lead to anovulatory phenomena, infertility and embryo loss (Kawano *et al.*, 2003 and Geva and Jaffe, 2000). Reduced follicular vascularity and reduced DNA synthesis have been described in atresic follicles (Greenwald, 1989). Studies have indicated that vascular endothelial growth factor (VEGF) may be involved in the physiological regulation of ovarian angiogenesis. Many factors influence VEGF production. Undoubtedly, hypoxia is the main stimulus for the VEGF synthesis and liberation and is able to rapidly induce its gene expression. LH and hCG in physiological conditions are powerful stimulators of the expression of mRNA for VEGF by the granulosa cells (Koos, 1995). Studies have demonstrated that VEGF is inadequate in stimulating the development of blood vessels if there are low insulin-like Growth Factor-I (IGF-I) levels (Smith *et al.*, 1999). Hellstrom *et al.* (2001) found that VEGF and IGF-I are complimentary in exercising their action on the endothelial cells, always through mitogen protein-kinase activity.
Also, the aim of this study was to evaluate, in dominant follicles close to ovulation, the follicular environment with particular attention to variations in VEGF concentrations.

Materials and Methods

The experiment was performed on seventeen multiparous Italian Friesian dairy cows selected from 500 cows of one herd. The selection was made on the basis of resumption of cyclic activity and delay from parturition. The animals used were 90 days postpartum and presented a good metabolic balance. No animal underwent a drop in body condition score (BCS) after parturition (100-(100*BCS one month postpartum/dry BCS)) greater than 20%. At 90-100 days, once the presence of regular luteal activity had been ascertained as described by Prandi *et al.* (1994) (Prandi *et al.*, 1994), each cow was synchronised by a double prostaglandin treatment (Veteglan, Calier, 0.15 mg im) at an interval of 11 d. At 72 hours, once luteolysis was effective , follicles with a diameter higher than 1 cm were subjected to ultrasound guided transvaginal aspiration. The follicular fluid was immediately collected in an EDTA tube. Blood samples were collected from the vena caudalis mediana into EDTA tubes immediately before each ultrasound-guided transvaginal follicular aspiration. The blood and the follicular fluid were centrifuged for 10 min at 3000 x g and the plasma and fluid obtained were divided into aliquots before storing at -20°C until required for analysis.
Progesterone (P4) and estradiol analysis were carried out using the RIA method described by Seren et al. (1974) (Seren *et al.*, 1974) and modified by Comin *et al.* (2002). IGF-I plasma concentrations were determined by the

radioimmunoassay method, as described by by Renaville *et al.* (1993). Follicular VEGF concentrations were determined by a heterologous radioimmunoassay method, as described by Comin *et al.* (2003). The recombinant mouse VEGF for iodination and standard curve preparation and the anti-mouse VEGF used were produced by Chemicon International, Inc The sensitivity of the analyses was 0.047 ng/ml. The efficiency of the method, within and between assays, was expressed by the coefficient of variation and resulted as 7.89% and 12.2%, respectively. The ED50 of the assay was 2.53 ± 0.25 ng/ml. The data values ranged between 0.05 and 1.5 ng/ml. Finally, plasma and follicular NEFA concentrations were measured with an enzymatic colorimetric kit (Wako Chemicals). Values were expressed in term of mean ± SEM. The differences between active and nonactive estrogen follicles were examined as unpaired groups. For a screening the unpaired t-test was applied to the grouped data of each variable.

Results

At 90°days post calving, all cows presented a BCS between 3.3 and 3.5.

The cyclic activity of the cows, monitored with progesterone analysis, was regular from 40±10 days after calving and remained regular for the entire experimental period. A drop in progesterone following the second prostaglandin treatment was apparent in all animals. Only the largest follicles were aspirated from each animal. The progesterone-estrogen ratio in the follicular fluid was used to divided follicles into two groups: the first included follicles with an average ratio of 9.58±3.07 (this group has been defined as estrogen active), while in the second, the average ratio was 0.16±0.08 (P=0.008) (defined as non active estrogen group). (Table 1). Average follicle diameter was 1.68±0.19 cm and 1.67±0.27 cm in the active and nonactive estrogen groups, respectively. The average estradiol concentration in the follicular fluid of the active estrogen group was 781.62±193.96 ng/ml, while in the nonactive, it was 28.40±15.43 ng/ml (P<0.01). The progesterone concentration did not show significant

Table 1: Average values of the considered parameters

	Group		
	Active estrogen	Nonactive estrogen	Difference
Number of follicle	10	7	NS
Diameter (cm)	1.68±0.19	1.67±0.27	NS
Estradiol/progres terone ratio	9.58±3.07	0.16±0.08	P<0.01
Plasma			
IGF-I (ng/ml)	87.83±20.19	39.01±5.87	P<0.05
NEFA (ng/ml)	157.26±36.87	265.28±87.95	NS
Follicular fluid			
Estradiol (ng/ml)	781.62±193.96	28.40±15.43	P<0.001
Progresterone (ng/ml)	118.14±34.8	137.57±31.68	NS
IGF-I (ng/ml)	90.12±17.77	35.08±6.79	P<0.05
NEFA (ng/ml)	129.94±19.50	431.93±89.50	P<0.001
VEGF (pg/ml)	664.10±79.64	942.86±110.37	P<0.05

differences in the two groups (118.14±34.80 active vs 137.57±31.68 ng/ml nonactive estrogen group) (Table 1). The endocrinological and metabolic profile was monitored 72 hours after the second prostaglandin treatment (day of follicular aspiration) by analysis of plasma IGF-I and NEFA. The plasma IGF1 concentrations in animals with active estrogen (87.83±20.19 ng/ml) and non active estrogen (39.01±5.87 ng/ml) follicles showed significant variation (P<0.05) (Table 1). The mean plasma NEFA concentrations (Table 1) did not differ significantly in the two groups (301.49±65.77 active vs 296.37±118.11 uEq/L non active estrogen group). The average VEGF follicular concentration increased significantly in the nonactive estrogen group (649.30±58.31 vs. 942.86±110.37 pg/ml; P<0.05). In the active estrogen group, the IGF-I concentration in the follicular fluid was significantly different from that in the nonactive estrogen group (90.12±17.77 vs 35.08±6.79 ng/ml; P<0.05). Finally, follicular NEFA levels (Table 1) increased significantly in the nonactive estrogen group (129.94±19.50 vs. 431.93±89.50 uEq/L; P<0.01).

Discussion

Only 59% of follicles displayed dominance characteristics (high estradiol content and high estradiol:progesterone ratio (Amiridis *et al.*, 1999). Large antral and pre-ovulatory follicles in cows are characterised by high intra-follicle estradiol levels and low progesterone levels, while atresic follicles contain higher levels of progesterone or androgen (Gwazdauskas *et al.*, 2000), as seen in subordinate follicles after selection of the dominant follicle or in the dominant follicle after loss of dominance (Mihm and Austin, 2002). The cows in this study were all in good

metabolic condition, as shown by the similar plasma levels of NEFA and IGF-I to those found in cattle with a positive energy balance at 70 days postpartum (Comin *et al.*, 2002). The presence of 41% follicles showing no dominance characteristics cannot therefore be blamed on an energy deficit.

In this study, VEGF concentrations in nonactive estrogen follicles were significantly higher. Studies on humans (Friedman *et al.*, 1998 and Quintana *et al.*, 2001) have demonstrated an inverse relationship between the follicular VEGF concentration and estradiol levels, total number of oocytes retrieved and embryo quality. There was instead a positive correlation with age and progesterone levels (Friedman *et al.*, 1998). These differences might be secondary to a diminished blood flow to the ovarian follicles that induces a compensatory increase in the production of VEGF in response to hypoxia (Kawano *et al.*, 2003; Friedman *et al.*, 1998 and Quintana *et al.*, 2001), or else to an initial luteinisation (Friedman *et al.*, 1998). VEGF is associated with follicles that display high vascularisation and oxygenation and oocytes with higher pregnancy potential (Kawano *et al.*, 2003), but high levels, the effect of the destruction of a delicate balance of vascular growth, might contribute towards a variety of reproductive disorders. In fact, studies on humans have demonstrated that an alteration in ovarian angiogenesis would lead to anovulation and infertility, or embryo loss (Quintana *et al.*, 2001 and Geva and Jaffe, 2000). Again according to these authors, high VEGF concentrations in the follicular fluid would be a marker of reduced gravidic potential.

The other hormonal parameter considered was IGF-I concentrations, which resulted as being similar in the plasma and follicular fluid, as also found by (Perk *et al.*, 1999), a sign that it might be transported from the blood (Gwazdauskas *et al.*, 2000). In fact, local production of IGF-I in the bovine follicle is absent or extremely low (Perk *et al.*, 1999). Therefore the effects of IGF-I in the ovary could be controlled more by variations in the local receptors or in the IGFBPs than by variations in the ovarian production of IGF-I. The significantly higher IGF-I concentrations found in active estrogen follicles may be explained by their involvement in the selection and differentiation of the dominant follicle or in follicular atresia (Echternkamp *et al.*, 1994). Follicular IGF-I levels increases as the estradiol and follicle diameter increase (Echternkamp *et al.*, 1994). The reduced follicular IGF-I secretion found in follicles with a low estradiol:progesterone ratio could alter the production of follicular estradiol and therefore the quality of the oocyte in the dominant follicle.

Regarding NEFA, the significantly higher concentrations found in the nonactive estrogen follicles might indicate a loss of energy in the follicles and their hypothetical involvement in the development of the dominant follicle. Higher follicular levels have also been found in cows subjected to fasting (Comin *et al.*, 2002 and Jorritma *et al.*, 2003). Jorritsma *et al.* (2003) found a similarity between plasmatic and follicular levels in heifers, but very little is known about the follicular concentrations of NEFA in cattle. There is more information available on pigs, where differences have been reported in plasmatic and follicular NEFA and in their concentration in dominant and subordinate follicles (Yao *et al.*, 1980).

Our data suggest that VEGF and NEFA, as well as follicular IGF-I, may play an role in follicular growth and could be valuable biochemical markers of oocyte maturation. It would be interesting to verify if the variations in VEGF, IGF and NEFA levels found in the dominant follicle close to ovulation might also occur at an earlier stage, during recruitment and selection. Discovering the best endocrine structure for correct development and follicle growth would perhaps help to better explain phenomena of hypofecundity in high producing dairy cows.

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