DOI : 10.5958/2277-8934.2021.00022.9 *Vol 28 No 2, p 131-135*

THE ACTIVITY OF GLYCOSIDASES (β -N-ACETYLGLUCOSAMINIDASE, α-N-ACETYLGALACTOSAMINIDASE AND α L FUCOSIDASE) IN THE UTERINE LUMINAL FLUID AND BLOOD SERUM OF THE DROMEDARY CAMEL (*Camelus dromedarius*) DURING THE FOLLICULAR CYCLE

I.M. Ghoneim[1,2], M.M. Waheed[1,2], A.M.A. Meligy[1,3] and S.M. El-Bahr[4,5]

[1]Department of Clinical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa, 31982, Saudi Arabia
[2]Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza 12515, Egypt
[3]Animal health Research Institute, Agricultural Research Centre, Giza, Egypt
[4]Department of Biomedical Sciences (Biochemistry), College of Veterinary Medicine, King Faisal University, Al-Ahsa, 31982, Saudi Arabia
[5]Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Egypt

ABSTRACT

The activity of three glycosidases (β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase) were estimated in the uterine fluid and serum throughout the follicular cycle of 24 slaughtered dromedary camels. These camels had ovaries bearing different size follicles (0.5-20mm) that grouped into group I (5–10 mm), group II (11–15 mm) and group III (16–20 mm). β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase were determined in the uterine fluid and serum samples using ELISA kits. Results indicated that mean concentration of β-N-acetylglucosaminidase in uterine fluid was 3.33 ± 0.26, 2.73 ± 0.15, and 6.43 ± 1.41 ng/ml in Group I, II, and III, respectively. The level of α-N-acetylgalactosaminidase in uterine fluid was 3.21 ± 0.27, 2.22 ± 0.14, and 7.24 ± 1.45 ng/ml in Group I, II, and III, respectively. α-N-acetylgalactosaminidase concentration showed statistical differences (P<0.05) among the different groups. The value of α-L-fucosidase in uterine fluid was 435.00 ± 6.94, 340.00 ± 9.82, and 362.50 ± 31.92 µmol/ml in Group I, II, and III, respectively. The maximum mean activity of α-L-fucosidase (P<0.05) was reported in-group I. In group I, the concentrations of β-N-acetylglucosaminidase and α-N-acetylgalactosaminidase were significantly (P<0.05) higher in the uterine fluid than the blood serum. In-group II, the concentration of α-N-acetylgalactosaminidase in the serum was significantly higher (P<0.05) than in the uterine fluid. In-group III, β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase concentrations were significantly (P<0.05) higher in uterine fluid than in the serum. The results proposed that β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase might play a role in carbohydrate-mediated events in the uterus of dromedary camels.

Key words: Camel; Uterine fluids; α L fucosidase, α-N-acetylgalactosaminidase; β-N-acetylglucosaminidase

Dromedary camels have been classified as induced ovulators, the ovulation mainly occurs in response to coitus (El-wishy, 1987; Ismail, 1987). In the absence of mating, the camel's oestrous cycle has no luteal activity (El-wishy, 1987; Skidmore *et al,* 1996), therefore, the oestrous cycle is described as a follicular wave pattern (Tibary and Anouassi, 1997; Skidmore *et al,* 2013). The duration of the follicular wave was recorded to extend from 17.2 to 30.0 days (Skidmore *et al,* 1996). The luminal fluids in the female reproductive tract are significant for mammalian reproduction as they provide the favourable microenvironment for passage of spermatozoa and ova, fertilisation and development of the pre-implantation embryo (Fischer and Beier, 1986; Velazquez *et al,* 2010). As the penis of the male camelids penetrates the cervical canal during copulation to deposit semen in the uterus (Vaughan and Tibary, 2006), the uterine luminal fluid is considered as initial microenvironment contacting the spermatozoa on their way to the oviduct. As the importance of uterine fluid, many studies have been investigated the different chemical constituents in

SEND REPRINT REQUEST TO M.M. WAHEED email: mmwaheed@kfu.edu.sa

cow (Forde *et al,* 2014; Tríbulo *et al,* 2019), ewe (Ko *et al,* 1991; Koch *et al,* 2010), saw (Zavy *et al,* 1984; Seo *et al,* 2012) and mare (Hayes e*t al,* 2012). Glycosidases are a group of hydrolytic enzymes originate from lysosome and catalyse the hydrolysis of glycoproteins, glycolipids and glycosaminoglycans (Miller *et al,* 1993; Hahn *et al,* 2001; Jóźwik *et al,* 2003). Glycosidases play an important role in different reproductive events such as cumulus cells expansion (Takada *et al,* 1994), sperm capacitation (Taitzoglou *et al,* 2007), sperm oviductal epithelial cells interaction (Lefebvre *et al,* 1997), sperm zona pellucida binding (Miranda *et al,* 2000; Zitta *et al,* 2006), polyspermy block (Miller *et al,* 1993) and early embryos development (Tsiligianni, 2018). The aim of the present study was to characterise the activity of β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α L fucosidase in the uterine luminal fluid and blood serum during the follicular phase of the dromedary camel (*Camelus dromedarius*).

Materials and Methods

Experimental materials

In the present study, one hundred genitalia were recovered from clinically healthy adult (7–16 years of age) non-pregnant female camels (*Camelus dromedarius*) during the breeding season (November–April), at a local abattoir in the Eastern province of Saudi Arabia. Blood samples (10 ml/ each animal) were collected from all animals during exsanguinations into non-heparinised tubes. Pre-slaughter reproductive history of these animals was not available. Genitalia and blood samples were kept in an ice box and transported immediately (within one-hour post slaughter) to the laboratory.

Collection of uterine fluid

Upon arrival at the laboratory, the genitalia with gross pathology or those with paired ovaries bearing follicles filled with sanguineous fluid or corpus luteum were discarded from the study. Depending on ovarian follicle diameter (measured by Vernier caliper), three groups of genitalia could be distinguished: Group l (genitalia have ovary/ ovaries bearing follicle/follicles of 5-10 mm), Group ll (genitalia have ovary/ovaries bearing follicle/follicles of 11–15 mm) and Group lll (genitalia have ovary/ ovaries bearing follicle/follicles of 16–21 mm). The uterine horns of each tract of different groups were longitudinally opened with surgical scissors from the cervix. The inner uterine mucus was aspirated from both horns using a positive displacement pipette suited for viscous media (Gilson MicroMan). Uterine fluid having cloudiness or with white flakes was excluded. Consequently, of one hundred collected genitalia only 24 were eligible for this study. The samples were distributed as: 8 in Group I, 8 in Group II and 8 in Group III. Uterine fluid was centrifuged at 1250-x g at 4°*C* for 10 minutes to remove the cell and cell debris and the supernatant was transferred into storage vials, identified and stored at -20°*C* until further analysis. Blood sera from selected animals were separated and stored at -20°*C* until analysis.

Estimation of biochemical constituents in uterine fluid and blood sera

Commercial ELISA diagnostic kits (My Biosource) were used for determination of camel β-N-acetylglucosaminidase (Catalog # MBS094638), camel α-N-acetylgalactosaminidase (Catalog # MBS053019) and camel α L fucosidase (Catalog # MBS092780) in the uterine fluid and blood serum samples. The procedures for analysis and calculation were adopted as recommended by the manufacturer.

Statistical analysis

The data analysis of biochemical constituents in uterine fluid and blood serum was carried out using a general linear model procedure and means were compared by least significant difference using SPSS 16.0 statistical software (2007).

Results

Results for concentrations of β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase in the uterine luminal fluid of dromedary camels during the follicular phase are displayed in Table1. The mean concentration of β-N-acetylglucosaminidase was 3.33 ± 0.26, 2.73 ± 0.15 and 6.43 ± 1.41 ng/ml in Group I, II and III, respectively. The level of α-N-acetylgalactosaminidase was 3.21 ± 0.27, 2.22 ± 0.14 and 7.24 ± 1.45 ng/ml in Group I, II and III, respectively. α-N-acetylgalactosaminidase concentration showed statistical differences (P<0.05) among the different groups. The peak concentration was recorded in-group III while the minimum concentration was reported in-group II. The value of α-L-fucosidase was 435.00 ± 6.94, 340.00 ± 9.82 and 362.50 ± 31.92 µmol/ml in Group I, II and III, respectively. The highest mean activity (P<0.05) was reported in-group I. Data presented in Table 2 demonstrates the mean concentrations of β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase in the blood serum of the dromedary camels during the follicular phase. The mean concentration of β-N-acetylglucosaminidase

was 2.00 ± 0.04, 3.07 ± 0.30 and 2.49 ± 0.13 ng/ml. in-group I, II and III, respectively. The peak concentration (P<0.05) was recorded in-group II. The mean concentrations of α-N-acetylgalactosaminidase was 1.81 ± 0.02, 3.29 ± 0.38 and 2.60 ± 0.28 ng/ml. There were statistical differences (P<0.05) among the groups. The peak concentration (P<0.05) was recorded in group II. The mean concentration of α-L-fucosidase was 560.00 ± 64.25, 310.00 ± 31.45 and 280.00 ± 8.24 µmol/ml in group I, II and III, respectively. The peak concentration (P<0.05) was recorded in group I. Table 3 displayed a comparison between the mean concentrations of β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase in the uterine luminal fluid and blood serum of the dromedary camel during the follicular phase. In-group I, the concentrations of β-N-acetylglucosaminidase and α-N-acetylgalactosaminidase were significant (P<0.05) higher in the uterine fluid than the blood serum. In-group II, the concentration of α-N-acetylgalactosaminidase in the serum was significant higher (P<0.05) than in the uterine fluid. In-group III, β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase concentrations were significant (P<0.05) higher in uterine fluid than in the serum.

Discussion

Glycosidases are engaged in several reproductive events such as cumulus cells expansion (Takada *et al,* 1994), sperm capacitation (Taitzoglou *et al,* 2007), sperm oviductal epithelial cells interaction (Lefebvre *et al,* 1997), sperm zona pellucida binding (Miranda *et al,* 2000; Zitta *et al,* 2006), polyspermy block (Miller *et al,* 1993) and early embryos development (Tsiligianni, 2018). Recently, glycosidases activity in genital tract luminal fluid is used as markers of embryo quality (Tsiligianni, 2018), embryo recovery rate (Reilas *et al,* 2000), superovulatory response (Tsiligianni *et al,* 2007). As in cattle (Tsiligianni e*t al,* 2007; Tsiligianni, 2018), sheep (Tsiligianni e*t al,* 2003; Samartzi *et al,* 2020) and mares (Reilas e*t al,* 2000), this study verified the activity of glycosidases (β-N-acetylglucosaminidase, α -N-acetylgalactosaminidase and α L fucosidase) in the uterine luminal fluid of the dromedary camel. The current study revealed obvious concentrations fluctuation of the studied glycosidases in relation to the follicle size. Parallel, glycosidases activities were described of change during different reproductive pattern such as oestrous cycle (in rats; Pizarro *et al,* 1984; ewes; Roberts *et al,* 1976b and mares; Reilas and Katila, 2002), post-partum period (in mares, Reilas and Katila, 2002) and pregnancy (in cows; Roberts and Parker, 1974). Fluctuations in glycosidase activity during the oestrous cycle are hormonally controlled (Hansen *et al,* 1985). The activity of glycosidases is recorded to be regulated by progesterone, oestrogens (Gladson *et al,* 1998; Buhi *et al,* 2000; Reilas and Katila, 2002) and pH of uterine luminal fluid (Carrasco *et al,* 2008). In cows, Mather (1975) reported cyclic pH changes of uterine fluid with the period of the cycle. The alterations of the glycosidases during the follicular cycle recorded in this study supposed its role in carbohydrate-mediated events (Rahi and Srivastava, 1983; Roy e*t al,* 1983; Carrasco *et al,* 2008). Similar with the results of Carrasco *et al* (2008) in porcine oviductal fluid, we reported maximum activities of β-N-acetylglucosaminidase and α L fucosidase in the uterine fluid at the late follicular phase. This study demonstrated significant higher concentrations of β-N-

Table 1. Concentrations (mean ± SEM) of β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase in the uterine luminal fluid of the dromedary camel during the follicular phase.

Enzymes	Group I (5–10 mm, n = 8)	Group II (11–15 mm, n = 8)	Group III (16–20 mm, n = 8)
β-N-acetylglucosaminidase (ng/ml)	3.33 ± 0.26^{ab}	2.73 ± 0.15^{a}	6.43 ± 1.41^{b}
α-N-acetylgalactosaminidase (ng/ml)	3.21 ± 0.27^{a}	2.22 ± 0.14^{b}	7.24 ± 1.45^{c}
α-L-fucosidase (µmol/ml)	435.00 ± 6.94^{a}	340.00 ± 9.82^{b}	362.50 ± 31.92^{b}

Means with different superscripts in the same row are different at $P<0.05$

Table 2. Concentrations (mean ± SEM) β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase in blood serum of the dromedary camel during the follicular phase.

Enzymes	Group I (5–10 mm, n = 8)	Group II (11–15 mm, n = 8)	Group III (16–20 mm, n = 8)
β-N-acetylglucosaminidase (ng/ml)	2.00 ± 0.04^{a}	3.07 ± 0.30^{b}	2.49 ± 0.13^{c}
α-N-acetylgalactosaminidase (ng/ml)	1.81 ± 0.02^{a}	3.29 ± 0.38^{b}	2.60 ± 0.28^{c}
α-L-fucosidase (µmol/ml)	560.00 ± 64.25^{a}	310.00 ± 31.45^{b}	280.00 ± 8.24^{b}

Means with different superscripts in the same row are different at $P<0.05$

Table 3. Comparison among concentrations (mean ± SEM) of β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase in the uterine luminal fluid and blood serum of the dromedary camel during the follicular phase.

Follicles sizes	Enzymes	Uterine fluid	Serum
Group I (5–10 mm, n = 8)	β-N-acetylglucosaminidase (ng/ml)	$3.33^{a} \pm 0.26$	$2.00^{b} \pm 0.04$
	α-N-acetylgalactosaminidase (ng/ml)	$3.21^{a} \pm 0.27$	$1.81^{b} \pm 0.02$
	α-L-fucosidase (μmol/ml)	435.00 ± 6.94	560.00 ± 64.25
Group II (11–15 mm, n = 8)	β-N-acetylglucosaminidase (ng/ml)	2.73 ± 0.15	3.07 ± 0.30
	α-N-acetylgalactosaminidase (ng/ml)	$2.22^{a} \pm 0.14$	$3.29^{b} \pm 0.38$
	α-L-fucosidase (μmol/ml)	340.00 ± 9.82	310.00 ± 31.45
Group III (16–20 mm, n = 8)	β-N-acetylglucosaminidase (ng/ml)	$6.43^{a} \pm 1.41$	$2.49^{b} \pm 0.13$
	α-N-acetylgalactosaminidase (ng/ml)	$7.24^{a} \pm 1.45$	$2.60^{b} \pm 0.28$
	α-L-fucosidase (μmol/ml)	$362.50^{a} \pm 31.92$	$280.00^{b} \pm 8.24$

Means with different superscripts in the same row are different at $P<0.05$

acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase in the uterine fluid than the blood serum in-group I and II. Likewise, Roberts and Parker (1974), Roberts *et al* (1976a) and Roberts *et al* (1976b) reported elevated activities of β-N-acetylglucosaminidase and α-L-fucosidase in uterine washings of bovine, human and sheep when compared with serum. This suggests that the enzyme present in uterine fluid comes mainly from within the uterus. Moreover, Hansen *et al* (1985) and Thie *et al* (1984, 1986) confirmed that luminal and glandular epithelium of the endometrium of pig mare, ewe and rabbit synthesised β-N-acetyl glucosaminidase and α-L-fucosidase.

Conclusions

In conclusion, uterine fluid displays glycosidase activity, with specific differences throughout the follicular cycle of dromedary camels, proposing that these enzymes play a role in carbohydrate-mediated events.

Acknowledgements

The authors would like to acknowledge the Deanship of Scientific Research at King Faisal University for their financial support (the research group support track grant no.160018).

References

Buhi WC, Alvarez IM and Kouba AJ (2000). Secreted proteins of the oviduct. Cells, Tissues, Organs 166:165-179.

Carrasco LC, Romar R, Avil´es M, Gadea J and Coy P (2008). Determination of glycosidase activity in porcine oviductal fluid at the different phases of the oestrous cycle. Reproduction 136:833-842.

El-wishy AB (1987). Reproduction in the female dromedary (*Camelus dromedarius*): a review. Animal Reproduction Science 82:587-593.

Fischer B and Beier HM (1986). Uterine environment in early pregnancy. In: Embryonic mortality in farm animals. Sreenan, J.M., Diskin, M.G. (eds). Martinus Nijhoff Publishers. pp 93-108.

Forde N, McGettigan PA, Mehta JP, O'Hara L, Mamo S, Bazer FW, Spencer TE and Lonergan P (2014). Proteomic analysis of uterine fluid during the pre-implantation period of pregnancy in cattle. Reproduction 147:575-87.

Gladson M, Srinivasan N, Malini T, Arunakaran J, Aruldhas MM and Govindarajulu P (1998). Interaction of estradiol, progesterone and corticosterone on uterine connective tissue degrading enzymes. Endocrine Research 24:89-103.

Hahn G, Malenica M, Müller WD, Taubert E and Petrak T (2001). Influence of post-mortem glycolysis on meat quality and technological properties of turkey breast. Pages 325-328 in Proceedings of XV European Symposium on the Quality of Poultry Meat, Kusadasi-Turkey. WPSA Turkish Branch, Ismir, Turkey.

Hansen PJ, Bazer FW and Roberts RM (1985). Appearance of β-hexosaminidase and other lysosomal-like enzymes in the uterine lumen of gilts, ewes and mares in response to progesterone and estrogens. Journal of Reproduction and Fertility 73:393-404.

Hayes MA, Quinn BA, Lillie BN and Cote O (2012). Changes in various endometrial proteins during cloprostenol-induced failure of early pregnancy in mares. Animal Reproduction 9:723-741.

Ismail ST (1987). A review of reproduction in the female camel (*Camelus dromedarius*). Theriogenology 28:363-371.

Jóźwik A, Śliwa-Jóźwik A, Bagnicka E and Kołątaj A (2003). The influence of selection on reaction to stress in mice. IX. Effect of dietary protein level on activity of lysosomal enzymes in liver and kidney. Jounal of Animal Breeding and Genetics 120:124-131.

Ko Y, Lee CY, Ott TL, Davis MA, Simmen RC, Bazer FW and Simmen FA (1991). Insulin-like growth factors in sheep uterine fluids: concentrations and relationship to ovine trophoblast protein-1 production during early pregnancy. Biology of Reproduction 45:135-142.

Koch JM, Ramadoss J and Magness RR (2010). Proteomic profile of uterine luminal fluid from early pregnant ewes. Journal of Proteome Research 9:3878-3885.

Lefebvre R, Lo MC and Suarez SS (1997). Bovine sperm binding to oviductal epithelium involves fucose recognition. Biology of Reproduction 56:1198-1204.

Mather EC (1975). "*In vivo*" uterine lumen pH values of the bovine. Theriogenology 3:113-119.

Miller DJ, Gong X and Shur BD (1993). Sperm require β-N-acetyloglucosaminidase to penetrate through the egg zona pellucida. Development 118:1279-1289.

Miranda PV., González-Echeverría F, Blaquier JA, Mahuran DJ and Tezón JG (2000). Evidence for the participation of β-hexosaminidase in human sperm-zona pellucida interaction *in vitro*. Molecular Human Reproduction 6:699-706.

Pizarro RA, de Courel HR, de Giraudo RH and de Martínez NR (1984). Glycosidases in the rat uterus during the oestrus cycle. Acta Physiologica et Pharmacologica Latinoamericana 34:271-276.

Rahi H and Srivastava PN (1983). Hormonal regulation of lysosomal hydrolases in the reproductive tract of the rabbit. Journal of Reproduction and Fertility 67:447-455.

Reilas T and Katila T (2002). Proteins and enzymes in uterine lavage fluid of postpartum and nonparturient mares. Reproduction in Domestic Animals 37:261-268.

Reilas T, Muhtinen M, Oksanen M and Katila T (2000). Relationship between embryo recovery rate and uterine lavage fluid composition in postpartum mares. Reproduction Nutrition Development 40:383-391.

Roberts GP and Parker JM (1974). An investigation of enzymes and hormone-binding proteins in the luminal fluid of the bovine uterus. Reproduction and Fertility 40: 305-313.

Roberts GP, Parker JM and Henderson SR (1976a). Proteins in human uterine fluid. Journal of Reproduction and Fertility 48:153-157.

Roberts GP, Parker JM and Symonds HW (1976b). Macromolecular components of genital tract fluids from the sheep. Journal of Reproduction and Fertility 48:99-107.

Roy SK, Sengupta J and Manchanda SK (1983). Histochemical study of β-glucuronidase in the rat uterus during implantation and pseudopregnancy. Reproduction and Fertility 68:161-164.

Samartzi F, Theodosiadou EK, Rekkas CA, Saratsi A, Lymberopoulos AG, Vainas E and Tsiligianni T (2020). Effect of equine chorionic gonadotropin on glycosidase activity in the reproductive tract of ewes, in relation to ovarian response and embryo yield. Small Ruminant Research 191, DOI: 106186

Seo H, Choi Y, Shim J, Kim M and Ka H (2012). Analysis of the lysophosphatidic acid-generating enzyme ENPP2 in the uterus during pregnancy in pigs. Biology of Reproduction 87:1-8.

Skidmore JA, Billah M and Allen WR (1996). The ovarian follicular wave pattern and induction of ovulation in the mated and non-mated one humped camel (*Camelus dromedarius*). Journal of Reproduction and Fertility 106: 185-192.

Skidmore JA, Morton KM and Billah M (2013). Artificial insemination in dromedary camels. Animal Reproduction Science 136:178-186.

SPSS (2007). Statistical Package for Social Science for Windows version 16.0. Chicago: SPSS Inc.

Taitzoglou IA, Kokolis AN and Killian GJ (2007). Modifications of surface carbohydrates on bovine spermatozoa mediated by oviductal fluid: a flowcytometric study using lectins. International Journal of Andrology 30: 108-114.

Takada M, Yonezawa N, Yoshizawa M, Noguchi S, Hatanaka Y, Nagai T, Kikuchi K, Aoki H and Nakano M (1994). PH-sensitive dissociation and association of beta-N-acetylhexosaminidase from boar sperm acrosome. Biology of Reproduction 50:860-868.

Thie M, Bochskanl R and Kirchner C (1984). Purification and immunohistology of a glycoprotein secreted from the rabbit uterus before implantation. Cell and Tissue Research 237: 155-160.

Thie M, Bochskanl R and Kirchner C (1986). Further characterization of the β-glycoprotein of the rabbit uterus. Biology of Reproduction 35:683-689.

Tibary A and Anouassi A (1997). Artificial breeding and manipulation of reproduction in camelidae. In: Hassan II, Rabat, Maroc, editors. Theriogenology in Camelidae: Anatomy, Physiology, BSE, Pathology and Artificial Breeding. Abu Dhabi: Abu Dhabi Printing and Publishing Company. pp 413-457.

Tribulo P, Balzano-Nogueira L, Conesa A, Siqueira LG and Hansen PJ (2019). Changes in the uterine metabolome of the cow during the first 7 days after oestrus. Molecular Reproduction and Development 86:75-87.

Tsiligianni T (2018). The possible role of certain glycosidase on cow reproduction Theriogenology Insight 8:21-32.

Tsiligianni T, Amiridis GS and Vainas E (2007). Activity of glycosidases (beta-N-acetyloglucosaminidase, alpha-mannosidase, and betagalactosidase) in the uterine luminal fluid of cows after multiple ovulation. Canadian Journal of Veterinary Research 71:300-304.

Tsiligianni T, Samartzi F, Amiridis GS, Koronaki A, Liapouras C and Vainas E (2003). Relationship between glycosidase activity in the uterine luminal fluid and superovulatory response of Chios ewes [abstract]. Reproduction in Domestic Animals 38:364.

Vaughan JL and Tibary A (2006). "Reproduction in female South American camelids: a review and clinical observations". Small Ruminant Research 61:259-281.

Velazquez MA, Parrilla I, Van Soom A, Verberckmoes S, Kues W and Niemann H (2010). Sampling techniques for oviductal and uterine luminal fluid in cattle. Theriogenology 73:758-767.

Zavy MT, Roberts RMand and Bazer FW (1984). Acid phosphatase and leucine aminopeptidase activity in the uterine flushings of non-pregnant and pregnant gilts. Journal of Reproduction and Fertility 72:503-507.

Zitta K, Wertheimer EV and Miranda PV (2006). Sperm N-acetylglucosanidase is involved in primary binding to the zona pellucida. Molecular Human Reproduction 12:557-563.