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STUDIES ON SOME BIOCHEMICAL CONSTITUENTS OF FOLLICULAR AND OVIDUCTAL FLUID IN DROMEDARY CAMEL (*Camelus dromedarius*)

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ABSTRACT

The culture media used for *in vitro* embryo production (IVEP) technology in camels have been adopted from ones used in other domestic animal species. In order to improve maturation, fertilisation, and embryo culture media and thereby IVEP, a need exists to determine the ionic composition of follicular and oviduct fluids in this species. Reproductive tracts were collected from a local abattoir immediately after slaughter and transported to the laboratory within 2 h in separate ice-chilled sterile plastic bags. Oviductal fluid was aspirated from individual oviducts using sterilised Drummond pipettes. The follicular fluid was aspirated in sterile syringes attached to 22 gauge hypodermic needles. The biochemical constituents of follicular and oviductal fluid were measured by an automatic chemistry analyser (Roche Hitachi 912) using kits, reagents and instructions provided by the manufacturer. The concentration of sodium in developing and mature follicles was lower than that of the serum and cystic follicles, which tended to be similar. The concentration of potassium, phosphate and lactate significantly ($P < 0.05$) decreased as the follicle size increased with the highest concentration in 5-10 mm follicles and lowest in >20 mm cystic follicles. No difference was observed in the concentration of calcium, magnesium and chloride between serum and follicular fluid from different size follicles. No difference was observed in the concentration of glucose in the follicular fluid from different size follicles, however, it tended to be higher when compared with serum.

The concentration of sodium was lower in the oviductal fluid when compared with serum during all the stages of follicular development. During the oestrous/receptive phase of the cycle when ovaries were with mature follicles, sodium concentration was higher ($P< 0.05$) when compared with the other stages of follicular development. Potassium and phosphate concentrations were very high in the oviductal fluid when compared with serum through all the stages, however, it was lower during the peri-ovulatory period when compared with other stages of the reproductive cycle. No difference was observed in the calcium and chloride concentrations during the different phases of oestrous/ follicular cycle, however, both of them were lower than that present in the serum. Magnesium levels were higher in the oviductal fluid compared to serum but did not differ during different follicular developmental stages. High concentrations of glucose and lactate were observed during all the stages of reproductive cycle in the oviductal fluid when compared with the serum.

Key words: Camel, follicular fluid, oviductal fluid

Development of *in vitro* embryo production technology (IVP) has been slow in camelids when compared with other domestic animal species. The culture media used for this species have been adopted from ones used in other domestic animal species. Therefore, a need exists to determine the ionic composition of follicular and oviduct fluids, which may lead to improved maturation, fertilisation and embryo culture media and thereby improve *in vitro* embryo production in this species. The most important factors regulating the production of embryos *in vitro* are the culture systems used for *in vitro* oocyte maturation and culture of embryos. The components of the culture media and culture conditions can affect and even modulate the meiotic regulation of mammalian oocytes (Downs and Mastropolo, 1997; Kito and Bavister, 1997). Culture conditions for *in vitro* maturation in other domestic species have been improved such that nowadays, a large percentage of oocytes successfully complete nuclear maturation (Krisher and Bavister, 1998; Eppig, 1991). Although, there are many studies on *in vitro* oocyte maturation and *in vitro* embryo production in dromedary camels from oocytes collected from slaughterhouse ovaries or by OPU from live donors, but only a limited number of studies have reported pregnancies and offspring produced from such embryos (Wani, 2021). The embryos produced

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through IVEP techniques have been reported to have a low cell numbers and lower pregnancy rates when compared to their *in vivo* produced counterparts (Vettical *et al,* 2019), which could mean improper culture media and conditions.

During *in vivo* oocyte maturation, besides meeting nutritional requirements of the developing oocyte, follicular fluid also maintains a proper environment for the maturation of the oocyte. The follicle, which is an avascular compartment within the mammalian ovary, is separated from the peri-follicular stroma by the follicular wall that constitutes a blood–follicle barrier (Bagavandoss *et al,* 1983). Within the ovarian follicle, the developing oocyte is surrounded by the follicular fluid, which is a serum transudate modified by follicular metabolic activities. Besides a serum transudate, follicular fluid also contains locally produced substances that share the metabolic activity of follicular cells (Gerard *et al,* 2002). Similarly, after fertilisation, the zygote/embryo is dependent on the nutrients provided by the oviduct and uterine fluids for its growth and survival (Ellington, 1991; Leese, 1995; Leese *et al,* 1985; Bavister, 2000). In particular, the ionic composition of these fluids has been shown to be important for early embryo development (Bavister, 2000; Grippo *et al,* 1992; Mathews *et al,* 1998; Leese, 1988). Oviductal fluid is produced by selective transudation of blood constituents and by secretion of specific constituents. Ions, which play an essential role in the formation of oviduct fluid, move through the epithelial cells of the oviduct and uterus into the lumen of the reproductive tract causing a concentration gradient which in turn causes an osmotic gradient providing the driving force to transport water by osmosis out of the epithelial cells into the oviduct or uterine lumen (Leese and Gray, 1985). Despite the importance of ions in oviduct, zygote and early embryo development there is little published information on the ion concentrations of oviduct in camelids.

This study was, therefore, carried out to investigate the biochemical composition of follicular and oviductal fluid in dromedary camel.

Materials and Methods

Collection of Oviductal fluid

Reproductive tracts were collected from she dromedary camels of unknown reproductive history from a local abattoir immediately after slaughter and transported to the laboratory within 2 h in separate ice-chilled sterile plastic bags in a thermos flask on ice (0.0–2.0°C). Reproductive tracts were grouped as per the status of follicular development on ovaries as under:

1. Developing follicles (5 to 10 mm),
2. Mature follicles (>10 to < 20 mm),
3. Cystic follicles (≥20 mm),
4. Corpus haemorrhagicum (Fresh corpora lutea with indications of fresh ovulation).

Ovaries were trimmed off the tracts and stored in thermos flask on ice for experiment 2. Oviductal fluid was then carefully aspirated from individual oviducts within a short period of time (5 min/sample) under aseptic conditions using sterilised Drummond pipettes (Drummond Scientific, Broomall, PA). The pipette tip was inserted gently in the lumen of the oviduct at the side of the fimbriae and the fluid was aspirated along the lumen toward the uterine end as a result of the negative pressure created by the release of the Drummond plunger. The fluid recovered was pooled separately for each group, centrifuged at 2500 g for 5 min to remove cellular debris and stored at -20°C for later analysis.

Collection of follicular fluid

The ovaries from experiment 1 were trimmed off the surrounding tissues and based on their diameters, follicles were categorised as:

Developing follicles (5 to 10mm),

Mature follicles (>10 to <20 mm) and

Cystic follicles (≥20 mm)

The follicular fluid was aspirated in sterile syringes attached to 22 gauge hypodermic needles in three different tubes depending on the size of follicle. The follicular fluid from the same group collected on a day was pooled, centrifuged, aliquoted and stored at –20°C until further analysis.

Collection of Serum

Before slaughter, jugular blood was collected randomly from 5 to 6 animals each time (N = 35) by sterilised 18-gauge hypodermic needles and syringes. The serum was harvested and stored in aliquots at –20°C for further analysis.

Estimation of biochemical constituents

The biochemical constituents of follicular and oviductal fluid were measured by an automatic chemistry analyser (Roche, Hitachi 912) using kits, reagents and instructions provided by the manufacturer. The analyser uses several operational systems to perform the required function. It includes control system, sampling system, reagent system,

photometric measuring system, cell rinse system and ISE system. The test principle is a colourimetric assay. The photometric measuring system detects colour or turbidity produced by the chemical reactions between reagents and analyte of interest in the sample, while in the reaction cells. This is capable of monochromatic and bi-chromatic photometry of end point; kinetic, ultraviolet and visible light chemistry determinations.

Statistical analysis

The data is presented as mean ± SEM. The concentrations of various biochemical constituents in the follicular fluid from different size follicles and serum were analysed by ANOVA with Fisher protected least significant difference test (MINITAB statistical software, Minitab ltd, CV3 2TE, UK). The concentrations of various biochemical constituents in the oviductal fluid collected from the reproductive tracts of different ovarian status were also analysed by ANOVA with Fisher protected least significant difference test.

Results and Discussion

The concentration of various biochemical constituents in the follicular fluid and the serum are summarised in table 1. The concentration of sodium in developing and mature follicles was lower than that of the serum and cystic follicles, which tended to be similar. The concentration of potassium, phosphate and lactate significantly ($P < 0.05$) decreased as the follicle size increased with the highest concentration in 5-10 mm follicles and lowest in >20 mm cystic follicles. No difference was observed in the concentration of calcium, magnesium and chloride between serum and follicular fluid from different size follicles. No difference was observed in the concentration of glucose in the follicular fluid from different size follicles, however, it tended to be higher when compared with serum.

The concentration of various biochemical constituents in the oviductal fluid are summarised in table 2. The concentration of sodium was lower in the oviductal fluid when compared with serum during all the stages of follicular development. During the oestrous/receptive phase of the cycle when ovaries were with mature follicles, sodium concentration was higher ($P< 0.05$) when compared with the other stages follicular development. Potassium and phosphate concentration was very high in the oviductal fluid when compared with serum through all the stages, however, it was lower during the peri-ovulatory period when compared with other stages of the reproductive cycle. No difference was observed in the calcium and chloride concentrations during the different phases of oestrous/follicular cycle, however, both of them were lower than that present in the serum. Magnesium levels were higher in the oviductal fluid compared to serum but did not differ during different follicular developmental stages. High concentrations of glucose and lactate were observed during all the stages of reproductive cycle in the oviductal fluid when compared with the serum.

Despite low fertility and high embryonic losses in camels, there is very little published information on the ion concentrations of follicular and oviductal fluid in this species. To the best of our knowledge this is the first study in which the ionic and biochemical

Table 1. Concentration of different biochemical constituents in the follicular fluid and serum of camel (*Camelus dromedarius*)

Follicular size	Sodium	Potassium	Calcium	Magnesium	Chloride	Phosphate	Glucose	Lactate
5.0 to 10 mm	137.7 ± 9.41^{b}	13.2 ± 0.71^{a}	2.4 ± 0.15^{a}	1.06 ± 0.07^{a}	108.2 ± 10.11	2.5 ± 0.15^{a}	4.9 ± 0.36^{a}	11.5 ± 0.77^{a}
>10 to < 20 mm	133.8 ± 5.87^{b}	8.30 ± 0.27^{b}	2.3 ± 0.12^{a}	0.9 ± 0.05^{a}	111.8 ± 8.39	2.0 ± 0.09^{b}	5.5 ± 0.28^{a}	6.7 ± 0.30^{b}
≥20 mm	149.3 ± 2.18^{a}	6.5 ± 0.12^{c}	2.3 ± 0.05^{a}	0.9 ± 0.06^{a}	119.5 ± 3.66	1.5 ± 0.08^{c}	4.6 ± 0.39^{ab}	5.2 ± 0.34^{c}
Serum	149.7 ± 1.4^{a}	4.3 ± 0.0^{d}	2.4 ± 0.05^{a}	1.3 ± 0.03^{a}	114.0 ± 0.0	1.40 ± 0.0^{c}	4.03 ± 0.07^{b}	2.9 ± 0.03^{d}

Values in same column with different superscripts differ significantly at $P < 0.05$.

Table 2. Concentration of different biochemical constituents in the oviductal fluid and serum of camel (*Camelus dromedarius*)

Ovarian status	Sodium	Potassium	Calcium	Magnesium	Chloride	Phosphate	Glucose	Lactate
Developing follicles	$81.3 \pm 4.9ac$	70.8 ± 2.3^{a}	1.1 ± 0.07^{a}	3.5 ± 0.13^{a}	61.0 ± 4.5^{a}	15.9 ± 0.61^{a}	20.9 ± 1.5^{a}	30.4 ± 0.75^{a}
Mature follicles	108.4 ± 5.48^{b}	60.8 ± 1.65^{b}	1.3 ± 0.05^{a}	3.6 ± 0.18^{a}	62.0 ± 4.46^{a}	13.6 ± 0.59^{b}	14.3 ± 0.83^{b}	32.2 ± 0.73^{a}
Corpus haemorrhagicum	91.0 ± 9.26^{c}	61.6 ± 1.51^{b}	1.06 ± 0.23^{a}	3.02 ± 0.24^{a}	73.4 ± 7.07^{a}	13.02 ± 0.54^{b}	13.7 ± 0.67^{b}	26.6 ± 0.97^{b}
Cystic follicles	70.9 ± 3.65^{a}	71.8 ± 4.27^{a}	1.08 ± 0.10^{a}	3.3 ± 0.17^{a}	66.3 ± 5.58^{a}	16.3 ± 1.34^{a}	16.8 ± 1.30^{b}	30.2 ± 1.89^{a}
Serum	149.7 ± 1.4^{d}	4.3 ± 0.0^{c}	2.4 ± 0.05^{b}	1.3 ± 0.03^{b}	114.0 ± 0.0^{b}	1.4 ± 0.0^{c}	4.03 ± 0.07^{c}	2.9 ± 0.03^{c}

Values in same column with different superscripts differ significantly at $P < 0.05$.

composition of oviductal fluid in camels determined. We observed a lower concentration of sodium in developing and mature follicles when compared with the cystic follicles and serum, which is similar to earlier studies (Zia-ur Rahman *et al,* 2008) in the same species. The concentrations of sodium in developing follicles in our study was similar to 137.05 ± 3.6 mEq/L observed in small follicles by these authors. Iwata *et al* (2004) also reported difference in sodium concentrations between fluids from small and large follicles in bovine. Serum sodium concentration was also higher than small follicular fluid in pigs (Chang *et al,* 1976) similar to our findings in camel.

A decrease in potassium concentration with the increase in follicle size was observed, similar to that in bovine (Iwata *et al,* 2004: Leroy *et al,* 2004) and swine (Chang *et al,* 1976). Higher potassium in small follicles compared with large follicles has been attributed to glucose utilisation, a process that leads to the transfer of potassium from extracellular fluid to intracellular sites (Chang *et al,* 1976). Perhaps the same is true for the camel as higher glucose concentration was observed in developing and mature follicles in our study.

No significant difference in the chloride concentration between different follicle groups and serum was observed in present study. These findings are in contrast to the observations of Leroy *et al* (2004) who reported a decrease in chloride concentrations with the advancement in follicular growth in dairy cattle and Zia-ur Rahman *et al* (2008) who also reported a lower chloride concentration in follicular fluid when compared with serum in camels. The chloride is known to initiate the LH-stimulated steroidogenesis in the chicken granulosa cells (Morley *et al,* 1991), human chorionic gonadotropin-stimulated steroidogenesis in oocytes of amphibians (Skoblina and Huhtaniemi, 1997) and steroidogenesis in adrenal glands by influencing the cAMP production (Cooke *et al,* 1999). Chloride ions are also responsible for enhancing the activity of angiotensin-converting enzyme, a metalloenzyme, which has been discovered in the follicular fluid of the porcine ovaries (Matsui and Takahashi, 2002).

Calcium concentration in developing and mature follicles was similar to that of the serum in our present study, which does not agree to the earlier findings (Zia-ur Rahman *et al*, 2008) in same species, where authors have reported a lower concentration in follicular fluid when compared with serum. Our observations are, however, in agreement with Iwata *et al* (2004) who reported that calcium concentration did not differ between the small and large follicles in bovine. Calcium is required for normal functioning of the granulosa cells (Leung and Steele, 1992) and steroidogenesis in granulosa cells during *in vitro* studies (Eckstein *et al*, 1986).

The concentration of phosphate was higher in the developing and mature follicles when compared with serum and cystic follicles in the present study. These observations were in total contrast to what has been reported in an earlier study in same species (Zia-ur Rahman *et al,* 2008) where authors have reported a lower concentration of phosphorus in follicular fluid when compared with the serum. We have also observed a decrease in the phosphate concentration with the increase in follicular size in contrast to earlier study (Zia-ur Rahman *et al*, 2008) where the authors reported an increase with the follicular growth. Phosphate is known to be a vital part of cAMP, as the second messenger in physiological action of steroid hormones (Hafez, 1993). The concentration of cAMP increases with the maturation of follicles in pigs (Chang *et al,* 1976). Thus, higher phosphorus concentration in developing and mature follicles can be related to a high demand of cAMP in the present study.

Higher concentration of glucose in follicular fluid when compared with serum was observed, which is in agreement with the findings of Zia-ur Rahman *et al* (2008). However, no significant difference in the glucose concentration of fluid from different size follicles was seen in the present study which is in contrast to the findings of Zia-ur Rahman *et al* (2008). Glucose being the major energy source for the ovary, might be the reason for its higher concentration in follicles. It seems that follicles have the ability to filter and reserve the high concentrations of glucose from blood for utilisation in their development. Gerard *et al* (2002) and Iwata *et al* (2004) observed a decrease in the glucose concentration in the fluid of pre-ovulatory follicles developing from a dominant follicle in mares and cows, respectively. However, in dairy cows Leroy *et al* (2004) observed an increase in the glucose concentration of follicular fluid with an increase in the follicle size. Our observations are, however, in contrast to their findings of low glucose concentration in follicular fluid when compared with the serum, which might be due to species differences. A higher concentration of lactate was observed in the fluid from follicles compared to the serum. There was a negative correlation between the lactate in follicular fluid and the follicular size. Lactate was higher

in small follicles because it might be the alternate sources of energy for the cells in follicles (Harlow *et al,* 1987; Leroy *et al,* 2004).

Sodium is the major cation present in oviductal fluid and in blood serum in camels. Sodium concentration was highest in the oviductal fluid of animals with mature follicles, which are receptive to males, but it was still lower than that of serum. The concentration in present study was lower than that reported in different phases of the oestrous cycle in cattle (Kenny *et al,* 2002; Grippo *et al,* 1992) and about 8 times lower than that reported in oviductal fluid of Alpacas (Apichela *et al,* 2015). Sodium is essential for blastocyst expansion because the formation of fluid and the resulting blastocyst expansion is dependent on the pumping of sodium into the blastocoel cavity by the Na+K+-ATPase sodium pump (Hobbs and Kaye, 1986; Brison and Leese, 1993). Oviduct potassium was affected by the ovarian follicular activity. Potassium concentrations in the oviduct in the persent study were higher during the follicular development stage while as it significantly decreases around the receptive phase when the ovaries have mature follicles or with a fresh ovulation. These concentrations were similar to those reported for human oviduct fluid (David *et al,* 1973; Lippes *et al,* 1972). Potassium concentration in the oviduct was 15 times higher than in serum. In another study on llama, oviductal fluid (Apichela *et al,* 2015) the concentration of potassium was half of what we have observed in our study. Earlier studies in cattle (Jordan *et al,* 1983) and human uterine fluid (Casslen and Nilsson, 1984) also reported to have the highest potassium concentrations during late luteal phase of the oestrous cycle. The significantly higher potassium concentrations in oviduct fluid when compared with blood serum recorded in the persent study agrees with the previous reviews (Leese, 1988 and Hunter, 1988) who suggested that the oviduct potassium concentration of most mammalian species is higher than blood values. A higher potassium concentration than in blood was found to be necessary to support the fertilising capacity of spermatozoa and the development of the murine preimplantation embryos (Roblero *et al,* 1990). Furthermore, high potassium concentrations were found to have a beneficial effect in a culture medium, based on human tubal fluid, for the development of human preimplantation embryos (Quinn *et al,* 1985). The higher potassium concentrations in oviduct fluid compared to blood suggests an active transport mechanism and/or an active secretion of potassium ions by the oviductal epithelium thus regulating the composition of the secreted fluids.

The concentration of chloride in the oviductal fluid was not affected by the ovarian activity in the present study. It was, however, lower than that of the serum and its secretion pattern was similar to what was observed for sodium. Our results are in agreement with Schultz *et al* (1971) who reported a similar concentration range in cows. Chloride is the major ion responsible for fluid secretion into the reproductive tract. For example, *in vitro* studies on rabbit oviduct tissue (Schultz *et al,* 1971; Gott *et al,* 1988) and studies on monolayers of bovine oviduct epithelial cells (Reischl *et al,* 2000) have shown that the net movement of chloride ions in the secretory direction causes an electrochemical gradient which is coupled to the transport of water into the lumen of the oviduct. An *in vivo* study on human uterine fluid composition (Casslen and Nilsson, 1984) also reported high chloride concentrations and concluded that this ion was actively involved in fluid secretion across the endometrium through absorptive and secretory activities.

Phosphate concentration in oviduct fluid was lower during the peri-ovulatory period when compared with the follicular development period, however, it was still 9-10 times more than the serum concentrations. Thompson *et al* (2000) reported an attempt to measure phosphate concentrations in bovine uterine fluid on day 7, however, they failed to do so as the concentrations were below the detection limit of their assay. There are no other published data on cattle oviduct or uterine phosphate for comparison.

Magnesium concentrations in oviduct fluid were similar in all the groups but higher than that of serum. Our observations were in contrast to another study on llama oviductal fluid where in authors mentioned a 10 times lower concentration (Apichela *et al,* 2015). The concentrations of oviduct magnesium recorded in the present study, however, reflect the concentration used in *in vitro* capacitation and acrosome reaction studies on bull spermatozoa (Parrish *et al,* 1988). Magnesium plays a major role in embryo and foetal development as foetal malformations had been reported to be linked to severe magnesium deficiencies (Jordan *et al,* 1983).

Calcium concentration in oviductal fluid was numerically lower than the serum concentrations, however, we did not observe any difference in its concentration in the fluid from tracts with developing,

mature, cystic follicles or even with fresh ovulations in contrast to an earlier report in cattle (Grippo *et al,* 1992), where authors reported maximum oviduct calcium concentrations at oestrus and ovulation. Our observed values were 5 times higher than what has been reported in a study on oviductal fluid of llama (Apichela *et al,* 2015). The oviduct calcium is considered to be essential for sperm viability as it enables the binding of oviduct proteins to spermatozoa (Lapointe and Sirard, 1996) and is essential for sperm capacitation, acrosome reaction and fertilisation processes in humans (Stock and Fraser, 1989).

Higher levels of glucose and lactate were observed in the oviductal fluid from all the groups when compared to serum in the present study. Lactate concentration was highest during the follicular development stage; however, it reduced significantly after ovulation. Our results are similar to those reported in women where Lactate (L (+) isomer) increases from 4.9 mM in the follicular phase to 10.5 mM at ovulation and then decreases to 6.2 mM in the luteal phase (Gardner *et al,* 1996). Interestingly, they also used a suction pipette to collect small volumes (0.5 µL) of luminal fluids from naturally cycling patients who were being investigated for infertility. Glucose levels decreased from about 21 mM during the follicular development to around 14 mM peri-ovulatory stage in the present study. Dickens *et al* (1995) also reported a decrease in glucose concentration from follicular phase to midcycle in the human tubal fluid. The trend is similar in our observations as well; however, the values are higher, which might be due to species differences. The decrease in glucose in oviduct fluid peri-ovulation is consistent with it being a major energy source of the oviduct for utilisation in secretory activity, and muscular and ciliary movement (Gardner *et al,* 1996).

In conclusion, the present study has quantified the *in vivo* concentrations of anions and cations in follicular and oviductal fluid of camels and compared them to blood serum concentrations. The physiological importance of the ionic composition of oviduct and follicular fluid is in the context of its influence on spermatozoa motility and function, oocyte quality and embryo viability and development. This information increases our understanding of the *in vivo* environment of the oocyte maturation, early embryo development, and provides valuable information that may lead to improved *in vitro* culture media and thereby improved *in vitro* embryo production in camelids.

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