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Biochemical Analysis of Female Mice Urine with Reference to Endocrine Function: A Key Tool for Estrus Detection

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Species-specific chemical signals released through urine, sweat, saliva and feces are involved in communication between animals. Urinary biochemical constituents along with pheromones may contribute to variation across reproductive cycles and facilitate to estrus detection. Hence, the present study was designed to analyze such biochemical profiles, such as proteins, carbohydrates, lipids, fatty acids, in response with steroid hormones such as estradiol and progesterone. The experimental groups were normal, prepubertal, ovariectomized, and ovariectomized with estrogen-treated female mice. In normal mice, the protein and lipid concentrations in urine were significantly higher in proestrus and estrus phases and the quantity of fatty acids was also comparatively higher in estrus. Furthermore, certain fatty acids, namely tridecanoic, palmitic and oleic acids, were present during proestrus and estrus phases, but were exclusively absent in ovariectomized mice. However, the carbohydrate level was equally maintained throughout the four phases of estrous cycle. For successful communication, higher concentrations of protein and specific fatty acids in estrus are directly involved. The significant increase in estradiol at estrus and progesterone at metestrus seems to be of greater importance in the expression pattern of biochemical constituents and may play a notable role in estrous cycle regulation. Thus, we conclude that the variations observed in the concentration of the biochemical constituents depend on the phase of the reproductive cycle as well as hormonal status of animals. The appearance of protein and specific fatty acids during estrus phase raises the possibility to use these as a urinary indicators for estrus detection.

Key words: protein, carbohydrate, lipid, fatty acids, ovariectomy, estrus, mouse

INTRODUCTION

Female reproductive physiology is a complex process and the macromolecules produced from females depend upon hormonal regulation and physiological status. Communicating at the time of ovulation and the co-ordination of sexual behavior appears to be an important task for the successful fertilization. The odors produced from females may vary according to the reproductive phase (Michael, 1975; O'Connell et al., 1981). All mammals excrete chemical signals to the external environment through urine, saliva, feces, and specialized scent glands (Vandenberg, 1999). Probably the concentration of these signals may vary according to the phase as well as status of the animal. Among the various sources in communication, urine is one of the chief sources involved in the signal-receptor system (Bimova et al., 2009). The male has more attraction to estrus female than non-estrus due to the presence of some specific compounds (Dominic, 1991; Archunan, 2009).

Some animals have permanent proteinurea due to the occurrence of abundant concentrations of protein, which serve as chemical signals among the animals and not as an indicator of disease. The Major Urinary Protein (MUP) present in mice is a member of the lipocalin protein family, which

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has a central cavity lining with tryptophan residues (Robertson et al., 1998). The cavity has the capacity to bind with volatile chemical signals delivered into the external environment (Novotny et al., 1999). The expression of this protein is comparatively weaker in females than males (Finlayson et al., 1968), but this does not mean that MUP is of minor importance for females. However, at the estrus phase the protein is found at maximum concentration during estrous cycle (Stopka et al., 2007). It clearly shows that the sex attractants produced in estrus females may be necessarily transported by this protein.

The pheromones and their carrier proteins are involved in the chemical communication apart from the lipid fractions. Brahmachary (1996) identified lipids from the marking fluid of the tiger and considered that it could be responsible for the characteristic odor of the animal. Fatty acid levels vary from one trimester to trimester in pregnant, as well as in lactating, bovine urine (Rameshkumar and Archunan, 2001; Rameshkumar et al., 2003). Mucopolysaccharide layer serves as chemical signal in Asian elephant (Rasmussen, 1998). Rameshkumar et al. (2000) revealed that the feeding habit and the breakdown of sugar may lead to production of many metabolites that in turn may possibly act as pheromonal signals.

The composition of the excretory products may vary according to the various reproductive phases. Males are more attractive to females in estrus than in all other phases (Dominic, 1991) due to the release of putative chemical signals. It is therefore strongly believed that the biochemical constituents of urine vary during estrous cycle. However, these profiles have not yet been evaluated. Further, the role of estrogen in the regulation of biochemical constituents in urine is not known.

The identification of the biochemical constituents of the urine in female mice during various reproductive phases will aid us in elucidating the functional aspects of these compounds in the estrus phase. Hence, the present investigation was carried out to evaluate the biochemical constituents such as proteins, carbohydrates, lipids, and fatty acids, and hormones such as estradiol and progesterone during various reproductive phases among the normal, prepubertal, and ovariectomized and estrogen-treated ovariectomized mice so as to use these constituents as an indicator of estrus.

MATERIALS AND METHODS

Test animals

The subjects of this study were adult female mice 24 ± 03.0 g (± S.D.) of Swiss strain. They were 8–10 weeks old and separately housed in polypropylene cages with 2 cm of rice husk as bedding material. The animals were maintained under controlled temperature, light and dark cycle (12 hr/12 hr), provided with pellet food (Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum*.

Experimental procedure

Twenty-four adult female mice were divided into three groups: normal (12 animals), ovariectomized (six animals) and ovariectomized and estrogen-treated (six animals). The prepubertal group consisted of six animals.

Ovariectomy and estrogen treatment

Twelve female mice were anesthetized with 60 mg/kg pentothal sodium (Abbot Lab Ltd., Mumbai, India) diluted in 0.9% saline and injected by IP. Ovariectomy was performed as described by Kannan and Archunan (1998). Six ovariectomized mice were allowed to recover for approximately two weeks and then treated with estradiol 0.1% gel (Sandrena Organon, Holland) over the flank region approximately 8–10 mg, once daily for 15 days after ovariectomy.

Estrous cycle determination

The estrous cycle was observed in all mice through the observation of vaginal smear (Archunan and Dominic, 1991). The smears were analyzed under light microscopy for the observation of three types of cells: leukocytes, eptithelial and cornified cells. A proestrus smear consists of predominance of nucleated cells while the estrus smear exhibit only cornified cells. The metestrus smear consists of equal proportion of leukocytes and cornified cells and diestrus smear consists of predominance of leukocytes. The assessment was done by a single technician at 07:00–08:00 a.m.

Sample collection

The urine sample was collected at all the reproductive phases from all the groups. While collecting the urine sample a gentle abdominal massage was applied and the animal was held over a watch glass. The samples were pooled and screened through cheesecloth or nylon mesh (16–120 μm) at the time of collection and stored at –20°C to be analyzed within a week.

Biochemical constituents

The biochemical constituents such as proteins, (Lowry et al., 1951) carbohydrates, (Dubois et al., 1956) and lipids, (Folch et al., 1957) were analyzed in all the urine samples.

Fatty acid profile

Five ml of urine sample was taken and mixed with saponification reagent. The tubes were tightly closed and kept for 30 minutes at 60°C in a water bath. 2 ml of methylation reagent was added to each tube and kept again in the water bath at 80°C for 20 minutes. Finally, a sufficient amount of extraction solvent (200 ml hexane + 200 ml of diethyl ether) was added to each tube, and then closed tightly, and shaken thoroughly for 10 minutes. About 2/3 of the organic phase (upper layer) containing the fatty acid methyl esters were transferred into screw cap glass vials. From each vial 1 μl of the fatty acid methyl ester (FAME) was injected into the Gas Chromatography (GC) column (Miller and Berger, 1985).

Blood collection and serum separation

The blood was collected in clean microcentrifuge tubes from mice at various reproductive phases by puncturing the jugular vein. The serum was separated from blood by centrifugation at 3000 rpm for 10 minutes and stored at 20°C for hormone assays.

Hormone assay

The hormones such as estradiol and progesterone were estimated in the serum sample of various reproductive phases by solid phase radioimmunoassay using standard kits (Alpco Diagnostics).

Statistical analysis

The results obtained in the present investigation were subjected to statistical analysis using SPSS statistical software (version 11.0) and Two-way ANOVA with Duncans test.

RESULTS

Protein levels

The urinary protein content significantly differed across the different reproductive phases of female. The estrus urine contained the highest level of protein, followed by proestrus phase. The metestrus and diestrus urine contained more or

less similar concentrations of protein. The ovariectomized mice excreted the lowest level of protein of all the phases. On estrogen treatment the ovariectomized mice excreted a higher level of protein. The prepubertal mice also excreted a notable concentration of protein (Table 1: Duncan's post-hoc analysis).

Carbohydrate levels

The carbohydrate content in urine significantly varied across various reproductive phases of female mice ($F = 4.660$, d.f = 9, 49; $P < 0.001$). A significantly higher level of total carbohydrate was found in metestrus phases than in other phases of estrous cycle. The lowest level of urinary carbohydrates was noted during the proestrus phase. Further, the ovariectomized mice exhibited a reduced level of total carbohydrates and the estrogen replacement caused a notable increase in carbohydrate excretion (Table 1: Duncan's *post hoc* analysis).

Table 1. Biochemical constituents in the urine of female mice during various reproductive phases. Values are expressed in Mean ± SE. Means in the same vertical column that are not marked with the same superscript (alphabets) letters are significantly different at α = 0.5 level (Duncan's test).

S.No.	Urine samples	Biochemical constituents mg/ml		
		Protein	Carbohydrates	Lipids
1	Proestrus	3.74 ± 0.13^{a}	67.6 ± 4.22^{b}	221.4 ± 16.04^{b}
2	Estrus	4.11 ± 0.05^{a}	75.6 ± 5.40^{ab}	279.8 ± 06.59^{a}
3	Metestrus	2.71 ± 0.31^{b}	76.4 ± 8.08^{ab}	157.8 ± 09.52^{d}
4	Diestrus	2.50 ± 0.22^{b}	63.0 ± 5.02^{b}	156.0 ± 07.72^{d}
5	Ovx	0.81 ± 0.08^{d}	66.8 ± 5.26^{c}	073.4 ± 08.34^{e}
6	Ovx + estrogen treatment	2.52 ± 0.19^{b}	81.0 ± 6.01^{ab}	195.2 ± 06.37^{c}
7	Prepubertal	1.73 ± 0.17^{c}	45.2 ± 5.37^{d}	070.8 ± 05.68^{f}

Lipid levels

A considerable amount of lipids was excreted and the concentration significantly varied among the phases of estrous cycle ($F = 72.647$, d.f = 9, 49; $P < 0.001$). The highest level of lipid was excreted during the estrus followed by the proestrus phase of the estrous cycle. The metestrus and diestrus animals excreted a lower concentration of lipids in urine. Removal of ovaries in mice caused a significant reduction in urinary lipids, in fact estrogen treatment to ovariectomized mice enhanced the urinary lipids excretion (Table 1: Duncan's *post hoc* analysis).

Fatty acid profile during the estrous cycle

The GC analysis showed that free fatty acids in urine varied qualitatively and quantitatively across the estrous cycle. Twenty different fatty acids were detected in the female urine sample; among them six fatty acids, namely lauric, tridecanoic, myristic, palmitic, palmitoleic and oleic acid were present in all four phases of the estrous cycle. But the concentration of all the six fatty acids varied considerably across the estrous cycle and three fatty acids namely tridecanoic, palmitic and oleic acid were notably higher in the estrus followed by proestrus phase. Pentadecanoic acid was present in higher concentration in estrus phase, absent in proestrus and little quantity was detected during diestrus and metestrus phase. The nondecanoic acid was present during proestrus and estrus, which was totally absent during metestrus and diestrus phase. Henecosanoic acid was present during all phases except estrus (Table 2).

Fatty acids in ovariectomized mice with estrogen treatment

The urine of ovariectomized group contained six fatty

Table 2. Fatty acid profile of female mice urine. Values are expressed in Mean ± SE. Means in the same vertical column that are not marked with the same superscript (alphabets) letters are significantly different at α = 0.5 level (Duncan's test).

Name of the Fatty acids	Fatty acids mg/g of lipid						
	Proestrus	Estrus	Metestrus	Diestrus	Ovx	Ovx + estrogen treatment	Prepubertal
Lauric acid	0.44 ± 0.08^{ab}	1.38 ± 0.03^{a}	0.69 ± 0.02^{b}	0.65 ± 0.01^{b}	–	–	0.04 ± 0.02^{c}
Tridecanoic acid	14.8 ± 0.22^{b}	16.3 ± 0.05^{a}	7.71 ± 0.07^{d}	0.05 ± 0.07^{fg}	2.34 ± 0.02^{e}	9.48 ± 0.57^{c}	0.53 ± 0.02^{f}
Myristic acid	0.03 ± 0.07^{b}	0.04 ± 0.03^{b}	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}	–	–	2.07 ± 0.65^{a}
Pentadeccanoid acid	6.04 ± 0.16^{a}	5.98 ± 0.18^{ab}	0.00 ± 0.02^{d}	2.03 ± 0.14^{b}	–	0.72 ± 0.01^{c}	–
Palmitic acid	6.12 ± 0.18^{b}	12.2 ± 0.25^{a}	2.43 ± 0.11^{c}	0.01 ± 0.02^{de}	1.11 ± 0.35^{cd}	5.62 ± 0.21^{b}	0.02 ± 0.01^{d}
Heptadecanoic acid	–	0.08 ± 0.01^{b}	–	0.01 ± 0.04^{c}	0.04 ± 0.02^{b}	0.24 ± 0.01^{a}	–
Stearic acid	2.02 ± 0.07^{a}	0.04 ± 0.09^{b}	0.07 ± 0.01^{b}	–	–	–	–
Nondecanoic acid	0.02 ± 0.01^{b}	0.02 ± 0.02^{b}	–	–	0.67 ± 0.01^{ab}	0.87 ± 0.05^{a}	0.87 ± 0.05^{a}
Henecosanoic acid	0.03 ± 0.02^{bc}	–	0.36 ± 0.03^{a}	0.01 ± 0.02^{b}	–	0.23 ± 0.01^{ab}	–
Behenic acid	–	–	–	–	0.60 ± 0.02^{b}	3.35 ± 0.52^{a}	0.43 ± 0.02^{b}
Tricosanoic acid	–	–	–	1.07 ± 0.05^{a}	–	–	–
Lignoceric acid	–	0.01 ± 0.05^{c}	–	0.04 ± 0.01^{b}	–	0.08 ± 0.02^{b}	3.05 ± 0.15^{a}
Palmitoleic acid	0.08 ± 0.02^{a}	0.09 ± 0.01^{a}	0.07 ± 0.02^{a}	0.01 ± 0.01^{ab}	–	–	–
Oleic acid	6.17 ± 1.05^{bc}	10.1 ± 1.85^{a}	3.11 ± 0.30^{c}	0.05 ± 0.01^{d}	3.06 ± 0.35^{c}	6.03 ± 1.25^{bc}	7.32 ± 0.56^{b}
Mysteric acid	–	0.39 ± 0.02^{b}	0.04 ± 0.01^{c}	1.05 ± 0.25^{a}	–	0.04 ± 0.02^{c}	–
Linolenic acid	–	–	–	2.36 ± 0.42^{a}	0.05 ± 0.01^{c}	0.74 ± 0.02^{b}	0.34 ± 0.12^{bc}
Cislinolenic acid	0.09 ± 0.01^{a}	–	–	–	–	–	–
Elaidic acid	–	2.06 ± 0.05^{a}	–	–	–	0.85 ± 0.01^{b}	–
Eicosanoic acid	0.22 ± 0.02^{a}	–	–	–	–	–	–

acids (tridecanoic, palmitic, nondecanoic, behenic, oleic, and linolenic acid). Among these oleic and behenic acids were present in higher concentrations. Interestingly, behenic acid was observed in the urine of ovariectomized mice, which was found to be absent in estrous cycle of normal females. Further, tridecanoic and palimitic acids, which were present in higher concentrations in normal females, but were found reduced drastically in the urine with complete absence of pentadecanoic, henecosanoic, lignoceric, mysteric and elaidic acids in ovariectomized mice. The estrogen treatment showed an increase in quality and quantity of fatty acids and presence of twelve fatty acids was noted in the estrogen-treated group. In addition, tridecanoic, oleic and palmitic acids were excreted in higher concentrations when compared to ovariectomized females. Further, behenic acid was present only in ovariectomized mice, whereas absent in estrogen-treated ovariectomized mice (Table 2).

Prepubertals

The prepubertal urine contains nine fatty acids in lower concentrations. Among them, the lignoceric and myristic acids were present at notable levels. The elaidic acid shown to be present in estrus female urine was absent in prepubertal urine. However, the concentration of tridecanoic acid was found high in proestrus and estrus, as compared to that of diestrus. The prepubertal urine contained a higher concentration of myristic acid when compared to other phases of estrous cycle (Table 2).

Hormone analysis

Estradiol

The estradiol concentration varied significantly across the estrous cycle of female mice ($F = 66.663$, d.f = 6, 34; $P < 0.001$). A higher level of estradiol concentration was reported during the estrus phase than in all other phases in the cycle. Next to the estrus phase, the estradiol concentration was significantly higher in proestrus phase, when compared to other phases. The lowest level of estrogen was noted in ovariectomized mice. In fact, estradiol replacement in ovariectomized mice showed an elevation in estradiol. In the case of prepubertal animals, a significantly lower level of estradiol was noticed as compared to that of all other phases with the exception of ovariectomized mice (Table 3: Duncan's post-hoc analysis).

Table 3. Hormonal profiles in female mice of various reproductive phases. Values are expressed in Mean ± SE. Means in the same vertical column that are not marked with the same superscript (alphabets) letters are significantly different at = 0.5 level (Duncan's test).

S.No.	Blood Samples	Hormone titers	
		Estrogen pg/ml	Progesterone ng/ml
1	Proestrus	2.03 ± 0.30^{b}	01.03 ± 0.19^{c}
2	Estrus	2.68 ± 1.37^{a}	02.84 ± 0.21^{b}
3	Metestrus	0.88 ± 0.16^{c}	13.05 ± 1.32^{a}
4	Diestrus	0.95 ± 0.08^{c}	03.10 ± 0.05^{b}
5	Ovx	0.31 ± 0.04^{d}	0.31 ± 0.05^{c}
6	Ovx + estrogen treatment	1.12 ± 0.20^{c}	0.34 ± 0.25^{c}
7	Prepubertal	0.41 ± 0.04^{d}	0.54 ± 0.06^{c}

Progesterone

The progesterone concentration was also found to be vary significantly across the estrous cycle ($F = 77.051$, d.f = 5, 29; $P < 0.001$). A significantly higher level of progesterone was noted during the metestrus phase, followed by diestrus phase, than in the proestrus and estrus period. Progesterone was higher during estrus phase when compared to proestrus phase of the estrous cycle. Similarly, the ovariectomized mice with estrogen treatment showed a lower level of progesterone. The prepubertals revealed considerably a higher level of progesterone than that of ovariectomized mice treated with estrogen (Table 3: Duncan's posthoc analysis).

DISCUSSION

The present study established the variation of biochemical constituents in urine among the estrous cycle of female. In general, mouse urine contains proteins usually at higher concentrations in male than in female (Achiraman and Archunan, 2006). In the present study, a notable amount of protein was present in the female urine of estrus phase. Since, some estrus specific pheromones are released through the urine, the high level of protein may be required to function as carriers for the ligands and convey the chemical signal. It has been reported that excretion of Major Urinary Protein in urine acts as pheromone carrier (Beynon and Hurst, 2004). It is therefore possible to suggest that the higher level of protein excreted from female at estrus is useful to communicate her conspecifics. Further, the male shows specific behavior and spent more time when exposed to female urine, but stopped by a partition (Amstislavskaya and Popova, 2004) and it suggested that the proteins specific to estrus phase exclusively involved in attraction of male. The nature of the protein is not identified in the present study, but the appearance of a higher level of protein during estrus seems to be noteworthy.

It has been reported that the nature of feeding habits may have a major impact on excretion of bio-molecules through urine (Galef, 1994; Rameshkumar et al., 2000). This may be the reason for a considerable release of carbohydrates in urine. For instance, alteration in diet changes the urinary odours of guinea pig and mice (Beauchamp, 1976; Schellinck et al., 1997). It is also observed that a minor component of Major Urinary Protein complex of the house mouse revealed a glycoprotein containing N-linked oligosaccharide (Ziegler et al., 1993). In this regard, the insignificant changes in carbohydrate content during the estrous cycle are probably due to the changes in hormonal level. Ma et al. (1995) identified that the high level of circulating estrogen stimulates the breakdown of glycogen and other materials into glucose in mammals, which will be utilized for energy in cells during the time of ovulation. Thus the reduction of carbohydrates in blood in turn released through urine.

Lipids in the urine are not certainly a waste product. Reports have come out regarding tiger, in which the urinary lipids can be used as fixatives (Poddar-Sarkar, 1996). The mechanism of fixing volatile molecules by "lipids" has been observed in *Tulpaia belangeri* (shrew) (Stralendroff, 1987). Our earlier study also indicated that lipids play a crucial role in the sexual attraction in rat, and vary considerably on basis of physiological status (Kannan et al., 1998). Due to their

distinctive properties, even the presence of small amount of lipids in urine describes the status of the animals (Burger et al., 2008). Despite their key protective effect, the present results revealed that the endocrinological status would have a major impact on the excretion of lipids, and hence, the greater expression noted in the estrus phase than that of other phases of estrous cycle.

Several lines of evidence suggest that the urinary fatty acids act in individual identification as well as sex attractant in certain mammalian species (Mattina et al., 1991; Poddar-Sarkar and Brahmachary, 1999). Similarly, in the present study as many as 20 fatty acids were detected. Among them, six (namely, lauric, tridecanoic, myristic, palmitic, palmitoleic and oleic acid) were present in all four phases of the estrous cycle, and three fatty acids viz., tridecanoic, palmitic and oleic acid were found in higher concentrations only during estrus followed by proestrus phase, suggesting that they may be involved in sexual attraction. Consistent with this, some volatile fatty acids namely, acetic, propionic, and butanoic acids, varied during menstrual cycle in humans, and these fatty acids reached a peak near the time of ovulation in humans (Michael, 1975).

Interestingly, ovariectomized mice show some deletion of fatty acids, and the estrogen replacement showed the reappearance of some fatty acids such as pentadecanoic, henecosanoic, lignoceric, mysteric and elaidic which are strongly suggested to be estrogen specific. This interference was further supported by Rameshkumar et al. (2000) reported that the tridecanoic, pentadecanoic and myristic acid are present predominantly in ovulatory phase and these fatty acids may act as estrus specific in bovine urine. It is known that the ovulatory phase contains higher levels of estrogen and the notion of present report increases the possibility of role of estrogen with the fatty acid excretion. It is additionally hypothesized that fatty acids along with other biochemical components of urine play a prominent role in opposite sex attraction.

The palmitic and oleic acid were seen in higher concentrations during proestrus, estrus phases and in ovariectomized plus estrogen-treated females, which clearly indicates that these compounds may also be estrogen-dependent. This finding is consistent with the report of Mattina et al. (1991), which showed that palmitic acid was excreted in the reproductive phase of bobcat urine and is certainly involved in sexual attraction of conspecifics. Later, Kannan and Archunan (1999, 2001) reported the presence of palmitic acid in preputial and flank glands of house rat. Moreover, Selvaraj (2002) suggested that the male rat (*Rattus rattus*) spent more time oriented to oleic acid than female revealed it as a sex attractant.

It is known that gonadal hormones have a rhythmic variation among the various phases of estrous cycle. A similar trend was noticed in the present study. Higher level of estradiol concentration was reported during the proestrus and estrus phase in the estrous cycle. The increase in the concentration of estradiol in ovulatory phase is consistent with earlier reports (Finn and Martin, 1970; Wallen, 1990). The role of estrogen in the activation and maintenance of reproductive behavior is well established (Breedlove, 1992).

The concentration of progesterone and estradiol increased during the preovulatory phase and attained a low level during the ovulatory phase (Day, 2004). In accordance with this view in the present study, progesterone levels were higher in the metestrus phase declining towards the diestrus phase. Progesterone release occurs in an episodic fashion in correlation with LH release, during mid and late luteal phase (Eisthen et al., 1987). Similarly, a surge of progesterone was observed in the late metestrus and early diestrus in laboratory mice (DeLeon et al., 1990). However, the role of progesterone and estradiol was not obviously reported, and the level of progesterone prior to the raise of estrogen may help to regulate the occurrence and intensity of estrus behavior (Hansel and Convey, 1983). Furthermore, the dynamic property of the hormones revealed in the present study has added knowledge not only on estrous cycle regulation, but also seems to serve as the control switch for biomolecular excretion through urine.

CONCLUSION

The present findings revealed that the protein and lipid level was significantly higher during estrus than in other phases. Further, the estrogen replacement also showed a similar trend. Likewise, specific fatty acids such as palmitic and oleic acids were excreted in higher amount at estrus as well as ovariectomized with estrogen treated groups. Thus, these findings suggested that the excretion of biomolecules in urine is linked to the hormonal status of the animals. Hence, the appearance of biochemical constituents can be considered as estrus indicators. Since, urine is the potent source of communication, this technology can be used as a non-invasive method in estrus detection.

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