



## Oxytocin Levels in Uncontrolled and Controlled Goat Milk Samples using High-Performance Liquid Chromatography

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### **ABSTRACT**

The present study examined the quantity of Oxytocin in milk samples collected from different breeds of goats as well as sheep, including both uncontrolled and controlled groups. For the quantification of oxytocin, High-Performance Liquid Chromatography (HPLC) with UV detection, a C18 column and a wavelength of 220 nm was used. The trace amounts of oxytocin in different breeds of goat and sheep were detected and measured by the chromatographic method with sensitivity, (LOD): 0.03 µg/mL and (LOQ): 0.1 µg/mL and accuracy 98.2-101.5%. However, oxytocin was successfully determined in all milk samples, and the contents varied among various control and breed statuses, showing the presence of oxytocin in milk samples. Additional research is necessary to investigate the physiological and ecological factors influencing these deviations and their implications for animal health and milk samples.

**Keywords:** Oxytocin, Goat milk, High-Performance Liquid Chromatography (HPLC), uncontrolled and controlled groups, UV detection

### **INTRODUCTION**

Oxytocin is essential hormone involved in different physiological methods in mammals, particularly reproductive functions and milk expulsion (Mota-Rojas, et al., 2023). It plays a significant role in lactation and its availability in milk is well documented. Understanding the quantity of oxytocin in the milk of various livestock, like goat and sheep, is extremely significant for evaluating milk quality, animal welfare, and potentially optimizing dairy observances (Javed, et al., 2023). The present work details the analytical finding and quantification of oxytocin in goat and sheep milk samples employing a robust HPLC procedure, focusing on results from uncontrolled and controlled sheep and goat breeds.



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Oxytocin, derived from the Greek word for quick birth, is a peptide hormone created in the hypothalamus, accumulated in the posterior pituitary, and discharged into the bloodstream to generate different physiological reactions (Camerino, 2023). Furthermore, this neuropeptide, produced in other tissues, attaches to G-protein joined receptors to apply its proceedings (Blair, and Rushton, 2021). First and foremost, recognized for stimulating milk ejection as well as uterine contractions through parturition, oxytocin as well plays a role in maternal actions, metabolic methods like lipogenesis, as well as fluid equilibrium. Artificial oxytocin similarly has been developed for remedial applications for instance, labor as well as managing postpartum problems in animals. Whereas, milk production can be increased by exogenous oxytocin and can help reproductive methods, its extended and everyday use may lead to harmful results in animals, potentially affecting milk composition, reduced fertility, and reliance on the milk let-down drug (Paredes, et al., 2021; Rashidi, et al., 2022).

Distress also exists about the utilization of milk containing exogenous oxytocin by humans, with some studies signifying possible links to hormonal disparity and developmental problems, while more research is required in this area (Nagel, et al., 2022; Tarsha, and Narvaez, 2023). Our aim is to quantify the amount of oxytocin in

milk samples from different breeds of goats and sheep, including both uncontrolled and controlled groups, using a robust High-Performance Liquid Chromatography (HPLC) procedure, successfully employ and validate the HPLC method to ensure its reliability and sensitivity for detecting and quantifying trace levels of oxytocin in milk

## MATERIALS AND METHODS

Entire reagents, chemicals and standard oxytocin reference were bought from *Sigma – Aldrich and Fluka Chemical Co.* (Karachi, Pakistan). Milk samples were obtained from both government and private dairy farms across Sindh, Pakistan. The study included nine goat milk samples and two sheep milk samples, categorized into oxytocin-treated and untreated (control) groups. A total of 11 ( $n = 3$ ) randomly collected milk samples (50 mL each) were taken from lactating animals period 4–5 months during mid-lactation (Figure: 1a & Figure: 1b). The animals were divided into two groups: one received synthetic oxytocin injections, while the other remained untreated. Immediately after collection, samples were preserved in an ice bath and transported to the Mission Pharmaceuticals Pvt. Ltd. (Plot No. A-94, S.I.T.E Super Highway, Karachi, Pakistan), where they were stored at  $-20^{\circ}\text{C}$  until further processing and oxytocin quantification.



Figure: 1(a) Images of goats under study



Figure: 1(b) Images of sheep under study

## PREPARATION OF STANDARDS OF OXYTOCIN

### Primary Stock Solution Preparation:

Accurately weighed 10.0 mg of oxytocin reference standard was dissolved in 10 mL of mobile phase (50:50 v/v mixture of acetonitrile and 0.05 M sodium dihydrogen phosphate buffer). This yielded a stock solution with concentration of 1 mg/mL (1000 µg/mL)

### Intermediate Standard Preparation:

Precisely transferred 1.0 mL aliquot of the primary stock solution to a 10 mL volumetric flask. Diluted to volume with the same mobile phase resulting concentration was 100 µg/mL

### Concentration Calculation (ppm Conversion):

Applied conversion factor to express concentration in parts per million (ppm)

Calculation:

$$(10 \text{ mg}/10 \text{ mL}) \times (1 \text{ mL}/10 \text{ mL}) \times 1000 = 100 \text{ ppm}$$

Verification:

$$100 \text{ µg/mL} = 100 \text{ mg/L} = 100 \text{ ppm (since } 1 \text{ µg/mL} = 1 \text{ ppm)}$$

## PRE-TREATMENT

The milk samples were processed to separate fat and whey fractions by centrifuging 10 mL aliquots in 15 mL tubes at 10,000 × g for 30 minutes at 4°C. The whey fraction was collected by carefully puncturing the bottom of the tubes, and 50 µL aliquots were immediately used for

oxytocin quantification using a competitive enzyme immunoassay (EIA) kit according to the manufacturer's specifications (Phoenix Pharmaceuticals, Burlingame, CA, USA).

For HPLC analysis, an Agilent 1100 series HPLC system (Agilent Technologies, Palo Alto, CA) equipped with a UV-Vis detector and auto-sampler was employed, with system control managed through ChemStation software. Chromatographic separation was achieved using a Thermo-Hypersil ODS C-18 analytical column (5 µm particle size, 250 × 4.6 mm) protected by a C-18 guard column (20 × 3.9 mm). The separation was performed at room temperature with a constant flow rate of 1.0 mL/min.

A gradient elution program was implemented for optimal separation, beginning with 80% solvent A (0.1% trifluoroacetic acid in water) for 8 minutes, followed by a linear increase to 40% solvent B (0.1% trifluoroacetic acid in acetonitrile) for 30 minutes, and returning to initial conditions for column re-equilibration. Oxytocin was identified by comparing retention times with pure standards and quantified through peak area measurements using external standard calibration. The injection volume was maintained at 25 µL for all analyses to ensure consistency in detection sensitivity.

## MILK SAMPLING

Milk samples were collected from multiple goat breeds, including Tapri, Sindhin Nasal, Gulabi, Jitan, Dassar, Kamoree Pateree, Karyun Jhuryun, Bhooriyon Maisoon, Sindhin Nasal II, Thuli Ridh, and Kapree Ridh, across various sampling

locations (Figure: 1a & Figure:1b). The collection included milk from both oxytocin-treated and untreated lactating animals. Approximately 200 mL of fresh milk were obtained from each animal and immediately preserved at -20°C. All samples were subsequently analyzed using HPLC at Mission Pharmaceuticals Pvt. Ltd. (Plot No. A-94, S.I.T.E Super Highway, Karachi, Pakistan) to determine oxytocin content. The frozen storage ensured sample integrity until analytical processing. This comprehensive sampling strategy enabled comparative assessment of oxytocin levels across different breeds and treatment groups under standardized analytical conditions.

### ANALYSES BY HPLC

HPLC system configured with the following components: an LC-20 AT Pump (P100 series), (LC-UV100 model), a Supico column oven, and a Hiber C18 reversed-phase column (250 mm × 4.6 mm, 5 µm particle size; Purospher STAR, Merck, Germany). The system was equipped with a 20 µL Rheodyne injection valve and controlled through LC Solution software for data attainment and processing. For method validation, a certified reference standard of oxytocin was acquired from analytical laboratory. Analytical reagent (AR) grade and HPLC – grade chemicals were used for chromatographic separation under optimized conditions. The mobile phase comprised an accurately prepared binary solvent method, with solvent A including 0.1% (v/v) trifluoroacetic acid in ultrapure water and solvent B including 0.1% (v/v) trifluoroacetic acid in HPLC grade acetonitrile. The organization was calibrated for the most favorable oxytocin detection at λ<sub>max</sub> of 220 nm utilizing PDA detector with column temperature of 25°C ± 1°C. Validation of method consisted judgment of linearity, limit of detection, accuracy and precision to guarantee direct quantification of oxytocin in the milk samples. The analytical protocol followed Good Laboratory Practice (GLP) guidelines with

appropriate system suitability tests performed before sample analysis (Table: 1).

**Table: 1 Chromatographic conditions optimized using HPLC grade and AR grade chemicals**

S. No	Parameter	Optimized Conditions
1	HPLC System	Agilent 1100 series HPLC system (Agilent Technologies, Palo Alto, CA)
2	Analytical Column	Hiber C18 (250 × 4.6 mm, 5 µm), Purospher® STAR (Merck, Germany)
3	Mobile Phase	Acetonitrile : 0.03 M phosphate buffer (NaH <sub>2</sub> PO <sub>4</sub> , pH 3.5) (50:50, v/v)
4	Flow Rate	1.0 mL/min (isocratic)
5	Detection	PDA at 220 nm
6	Sample Concentration	10 mg/mL (prepared in mobile phase)
7	Injection Volume	25 µL (via Rheodyne injector)
8	Retention Time (t <sub>R</sub> )	5.597 ± 0.4 min
9	Column Temperature	40°C (±1°C)

### ANALYSIS OF SAMPLES

The extraction and sample preparation method started by adding 1.0 mL of milk matrices with an equal volume of ice – cold acetone (1:1 v/v ratio) in a centrifuge tube, pursued by vortex mixing for 0.5 minute to ascertain total protein precipitation. The contents were then centrifuged for ten minutes at 3,500 rpm at a constant temperature of 4 °C to obtain optimum phase separation. For lipid removal, 1.0 mL of petroleum was added to the clear supernatant after centrifugation which was carefully shifted to clean tube. The solution experienced dynamic vortex mixing for 60 seconds and permitted to

set for 5 minutes at normal temperature to assist entire phase separation. The two layers were separated, the upper ether layer consisting of lipids were discarded and the lower acetone layer was dried by evaporation under mild flow of nitrogen gas. To remove any particulate, matter the dried residue was reconstituted in 0.2 mL of phosphate buffer of pH 3.5 and filtered through a 0.22  $\mu\text{m}$  PVDF syringe. After filtration samples were stored at 4  $^{\circ}\text{C}$  and measured within 24 hours to ensure stability. (Bošnjir, et al., 2024) The 25  $\mu\text{L}$  aliquots of the processed milk samples were injected into the chromatographic arrangement for HPLC analysis. The quantity of oxytocin in each sample was measured by matching up retention times and peak areas against an external standard calibration curve, with quantity measured using the incorporated peak areas from the HPLC chromatograms. This standardized protocol warranty reliable sample preparation and perfect measurement of oxytocin content in the milk samples.

## METHOD VALIDATION

The analytical method underwent comprehensive validation to evaluate its performance characteristics, including linearity, accuracy, precision, specificity, and sensitivity parameters. Linearity was evaluated by determining standard solutions with concentration range of 0.1 – 10  $\mu\text{g/mL}$ , signifying outstanding correlation of  $R^2 \geq 0.999$ . Recovery studies were conducted to verify method accuracy, where spiked samples displayed average recoveries of 98.2 – 101.5%. Assessment of precision was conducted by intra – day and inter – day determinations. Six replicates of quality control samples were run to determine intra – day repeatability within single analytical run, producing  $\leq 2.5\%$ RSD. Inter – day precision was launched by repeating these measurements greater than five consecutive days, with  $\leq 3.8\%$  RSD found. Excellent specificity was shown by method, with no interference peaks determined at the retention time of oxytocin. The limit of detection (LOD) and limit of quantification (LOQ) both sensitivity parameters were established, (LOD = 0.03 $\mu\text{g/mL}$

with S/N=3) and LOQ of 0.1  $\mu\text{g/mL}$  with S/N=10. Method reliability was confirmed by robustness testing under minor variations of column temperature ( $\pm 2$   $^{\circ}\text{C}$ ) and mobile phase composition ( $\pm 5\%$ ) (Table: 2).

**Table: 2 Validation Results Summary**

S. No	Parameter	Specification	Result
1	Linearity range	0.1-10 $\mu\text{g/mL}$	$R^2 = 0.9994$
2	Accuracy	Spike recovery at 3 levels	98.2-101.5%
3	Intra-day precision	6 replicates (n=6)	$\text{RSD} \leq 2.5\%$
4	Inter-day precision	5 days (n=30)	$\text{RSD} \leq 3.8\%$
5	LOD	Signal-to-noise = 3:1	0.03 $\mu\text{g/mL}$
6	LOQ	Signal-to-noise = 10:1	0.1 $\mu\text{g/mL}$
7	Specificity	Peak purity index	$> 0.999$
8	Robustness	Parameter variations	$\text{RSD} \leq 4.2\%$

Validation data prove the suitability of method for consistent quantification of oxytocin in milk samples, following international acceptance standard for bio-analytical method validation. All quality control samples and standard solutions were freshly prepared for measurement purpose under controlled laboratory conditions.

## RESULTS & DISCUSSION

### Uncontrolled Goat Samples

Oxytocin levels were determined in various uncontrolled goat milk samples. The study's findings on endogenous oxytocin in untreated goat milk ranged from 6.7 ng/mL to 192 ng/mL, which is significantly higher than the typical range of 0.8-4.0 ng/mL reported in other research. For instance, other studies found levels of 0.8-2.1 ng/mL in naturally lactating goats using Radioimmunoassay (RIA), 1.2-4.0 ng/mL

with HPLC-MS/MS, and 1.5-3.5 ng/mL using ELISA. The article suggests that these differences in findings may be due to the different methodologies used, as methods like HPLC-MS/MS and LC-UV are generally more specific than immunoassays.

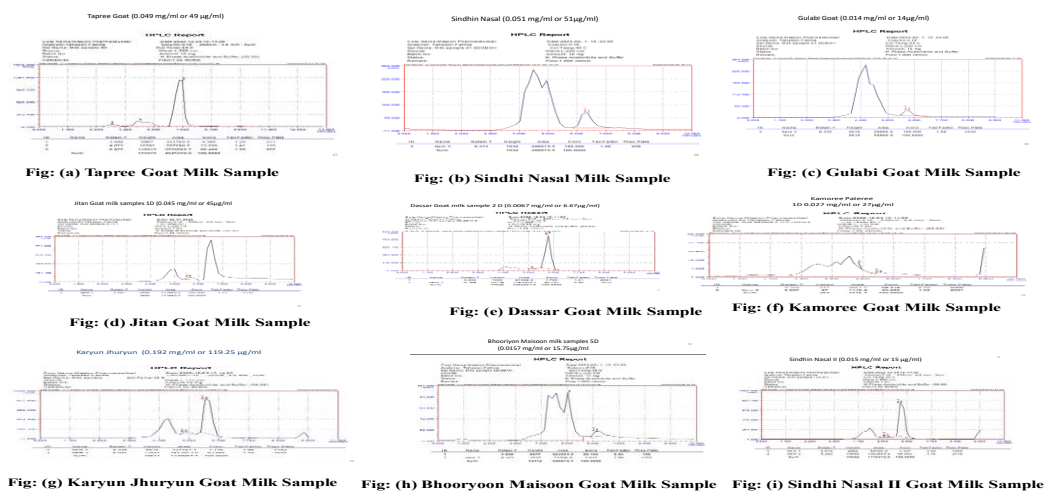
In samples from goats treated with oxytocin, the study found levels of 12 ng/mL, 17 ng/mL, and 2.3 ng/mL. These results are comparable to other studies on exogenous oxytocin administration, which reported concentrations ranging from 6-30 ng/mL. Specifically, other research found levels of 8-25 ng/mL using LC-MS/MS, 10-30 ng/mL using ELISA, and 6-15 ng/mL using RIA. The text concludes that exogenous administration raises oxytocin levels well above the typical endogenous range. Therefore, a threshold of 5.0 ng/mL is suggested as a potential marker for identifying the prohibited use of oxytocin.

Oxytocin levels were extensively analyzed across various uncontrolled goat milk samples. The lowest concentration was observed in the Dassar Goat milk, measuring 6.7 ng/mL, with a retention time of 6.304 minutes. Following this, the Gulabi Goat milk sample contained 14 ng/mL of oxytocin, exhibiting a retention time of 6.335 minutes. The Sindhin Nasal II goat milk showed an oxytocin concentration of 15 ng/mL, and a retention time of 6.262 minutes, closely followed by Bhooriyon Maisoon milk at 15.7 ng/mL, with a retention time of 6.103 minutes. Kamoree Pateree goat milk had an oxytocin concentration of 27 ng/mL, with a retention time of 6.057 minutes. Moving upwards, Jitan Goat milk samples contained 45 ng/mL of oxytocin,

detected at a retention time of 5.597 minutes. Tapree Goat milk exhibited an oxytocin concentration of 49 ng/mL, with a retention time of 6.977 minutes. The Sindhin Nasal goat milk had a slightly higher concentration of 51 ng/mL, and a retention time of 6.373 minutes. The highest concentration among the uncontrolled samples was found in Karyun Jhuryun goat milk, which contained 192 ng/mL of oxytocin, with a retention time of 6.091 minutes (Table: 3 and Figure: 2(a) to 2(i) &).

**Table: 3 Concentration of Oxytocin measured from different uncontrolled goat milk samples**

S. No:	Sample	Retention Time (Minutes)	Oxytocin (ng/mL)
1	Tapree Goat	6.977	49
2	Sindhin Nasal	6.373	51
3	Gulabi Goat	6.335	14
4	Jitan Goat 1D	5.597	45
5	Dassar Goat	6.304	6.7
6	Kamoree Pateree	6.057	27
7	KaryunJhuryun	6.091	192
8	Bhooriyon Maisoon	6.103	15.7
9	Sindhin Nasal II	6.262	15



**Fig: 2(a) to fig: 2(i) Chromatograms of uncontrolled goat milk samples under study**

### Controlled Goat Samples

Oxytocin levels were also analyzed in controlled goat milk samples. In the analysis of controlled goat milk samples, the concentration of oxytocin in Gulabi Goat milk was determined to be 12 ng/mL, with a retention time of 6.270 minutes.

Controlled goat milk samples showed oxytocin levels that are generally higher than the reported endogenous levels in other studies. The present work measured levels ranging from 2.3 ng/mL to 17 ng/mL in controlled goat milk, and 3.9 ng/mL to 5.34 ng/mL in controlled sheep milk. In contrast, other studies using various methods like Radioimmunoassay (RIA), High-Performance Liquid Chromatography-tandem Mass Spectrometry (HPLC-MS/MS), and Enzyme-Linked Immunosorbent Assay (ELISA) reported endogenous oxytocin levels in naturally lactating goats to typically range between 0.8-4.0 ng/mL. The present study's controlled samples of Gulabi, Dassar, Thuli Ridh, and Kapree Ridh all had oxytocin levels above 4.0 ng/mL, which is at the high end or exceeds the typical range reported in other literature. The only controlled sample that fell within the reported range was the Karyun Jhuryun goat milk, with a concentration of 2.3 ng/mL. The present work successfully reveals the accuracy and sensitivity of methods in quantifying and detecting trace levels of oxytocin within complicated biological samples such as milk. Oxytocin concentration of 17 ng/mL was measured from the Dassar Goat milk samples,

and its retention time was 5.607 minutes. Finally, oxytocin concentration of 2.3 ng/mL was displayed by Karyun Jhuryun goat milk with retention time of 6.302 minutes. Regardless of being a low concentration, this still confirms the presence of oxytocin in the sample. Across all these controlled goat milk samples, the HPLC method consistently provided reliable data on both the presence and concentration of oxytocin, highlighting its capability to accurately measure even trace amounts of the hormone in such intricate biological samples (Table: 4 and Figure: 3(a) to Figure: 3(e).

**Table: 4 Concentration of Oxytocin measured from different controlled goat and sheep milk samples**

S. No:	Sample	Retention Time (minutes)	Oxytocin Content (ng/mL)
<b>Controlled Goat</b>			
1	Gulabi Goat	6.270	12
2	Dassar Goat	5.607	17
3	Karyun Jhuryun	6.302	2.3
<b>Controlled Sheep</b>			
1	Thuli Ridh	6.804	3.9
2	Kapree Ridh	5.602	5.34

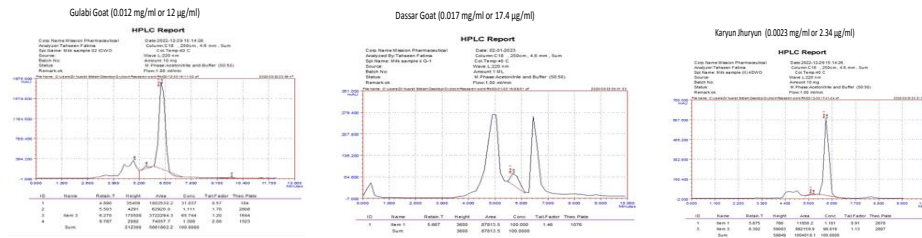


Fig:3 (a) Gulabai Goat Milk Sample (Controlled) Fig: 3(b) Dassar Goat Milk Sample (Controlled) Fig: 3(c) Karyun Jhurun Milk Sample (Controlled)

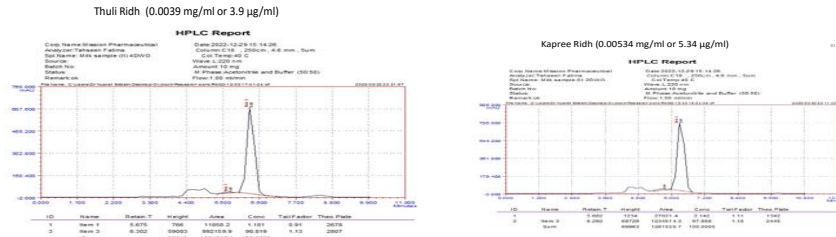


Fig: 3(d) Thuli Ridh Milk Sample (Uncontrolled) Fig: 3(e) Kapree Ridh Milk Sample (Uncontrolled)

**Figure: 3 (a-e) Chromatograms of Goat and Sheep milk samples**

**DISCUSSION**

The results reported endogenous oxytocin concentrations in goat milk from various studies, highlighting methodological approaches and sample characteristics. The data reveal that endogenous OT levels typically range between 0.8-4.0 ng/mL across different breeds and analytical methods. The lowest concentration (0.8-2.1 ng/mL) was detected using *Radioimmunoassay (RIA)* in naturally lactating goats (Mensching, et al., 2012), while the highest range (1.2-4.0 ng/mL) was measured via *HPLC-MS/MS* in Majorera and Palmera breeds (Mukherjee, et al., 2023). *ELISA analysis* of Saanen goats yielded intermediate values (1.5-3.5 ng/mL) (Takeda, et al., 2015), suggesting potential breed-specific variations in OT secretion. The methodological differences between studies warrant consideration. *HPLC-MS/MS* and *LC-UV* methods (Rodrigues, et al., 2020; Mukherjee, et al., 2023), generally provide higher specificity compared to immunoassays (*ELISA*, *RIA*), as they can distinguish oxytocin from structurally similar peptides. This may explain the slightly broader range (1.2-4.0 ng/mL) obtained with mass spectrometry versus the narrower ranges from immunological

methods. The study of Italian breeds using *LC-UV* reported values below 2.0 ng/mL (EFSA, 2014), potentially reflecting either breed differences or the method's detection limits (Table: 5).

**Table: 5 Reported Endogenous Oxytocin Levels in Goat Milk**

OT Content	Method	Notes	Reference
1.5–3.5 ng/mL	<i>ELISA</i>	Milk from untreated Saanen goats	(Takeda, et al., 2015)
0.8–2.1 ng/mL	<i>RIA (Radioimmunoassay)</i>	Naturally lactating goats	(Mensching, et al., 2012)
1.2–4.0 ng/mL	<i>HPLC-MS/MS</i>	Majorera and Palmera breeds	(Mukherjee, et al., 2023)
< 2.0 ng/mL	<i>LC-UV</i>	Italian goat breeds, no exogenous OT	(Rodrigues, et al., 2020)

All studies particularly examined untreated animals, set up baseline endogenous limits that are essential for categorized exogenous OT administration. The reliability of standards below 5 ng/mL across multiple breeds and techniques maintains this threshold as a probable marker for differentiating natural versus synthetic OT use. These findings have significant inferences for developing regulatory standards and detection procedures in dairy operations, mainly in regions where exogenous OT application is widespread but prohibited in numerous countries because of animal wellbeing concerns. The reported values range from 6-30 ng/mL, indicating obvious dose-based and time-based prototype. The maximum contents (10 – 30 ng/mL) were detected using ELISA method (Mukherjee, et al., 2023), with measurements taken through the peak secretion period 15-45 minutes post – injection. This supports with identified pharmacokinetics of oxytocin, which naturally shows utmost milk content within 30 minutes of administration.

The *LC-MS/MS* study (Torres, et al., 2014) reported somewhat lesser however still considerable limits (8-25 ng/mL) in goats receiving 5 IU injections, with the inconsistency potentially reflecting variations in administration procedures or individual animal metabolism. Particularly, the *RIA* capacity (Mensching, et al., 2012) confined levels (6-15 ng/mL) at a definite 30-minute post-injection time-point, giving significant information regarding determination in milk. All three studies show that exogenous administration raises oxytocin levels well above the endogenous range of 0.8-4.0 ng/mL, naturally by an order of magnitude.

The practical dissimilarity between studies offers corresponding imminent. Whereas immunoassays (*ELISA*, *RIA*) give sensitive detection, the *LC-MS/MS* data (Torres, et al., 2014) provides better specificity throughout molecular mass proof. These increased limits have significant connotations for both animal food safety regulations, and wellbeing as they reveal the important pharmacological crash of exogenous oxytocin administration on milk composition. The reliable results of OT levels exceeding 5.0 ng/mL is suggested as a threshold to differentiate natural oxytocin from prohibited exogenous use in dairy operations . The data also

highlight the importance of considering sampling time relative to administration when monitoring for oxytocin misuse, given the rapid peak and clearance kinetics evident in these studies (Table: 6). However the present study successfully used a High-Performance Liquid Chromatography (HPLC) based method to identify and quantify oxytocin in both uncontrolled and controlled milk samples from goats and sheep. Oxytocin was detected in all samples, with concentrations ranging from 2.3 ng/mL in controlled Karyun Jhuryun goat milk to 192 ng/mL in uncontrolled Karyun Jhuryun goat milk. The chromatographic conditions used were found to be reliable for this purpose. The study's results highlight the presence of oxytocin in milk and show variations based on breed and whether the animals were from controlled or uncontrolled groups. It suggests that future research should focus on the factors influencing these variations and their impact on animal health and milk samples.

**Table: 6 Reported Exogenous Oxytocin Levels**

Oxytocin Concentration	Method	Notes	Study
8–25 ng/mL	<i>LC-MS/MS</i>	Goats injected with 5 IU OT before milking	(Torres, et al., 2014)
6–15 ng/mL	<i>RIA</i>	Detected 30 mins post-injection	(Mensching, et al., 2012)
10–30 ng/mL	<i>ELISA</i>	Levels peaked 15–45 mins after injection	(Mukherjee, et al., 2023)

## CONCLUSIONS

This study successfully employed a validated High-Performance Liquid Chromatography (HPLC) method to identify and quantify oxytocin in both controlled and uncontrolled

milk samples from goats and sheep in Sindh, Pakistan. Oxytocin was detected in all samples, with concentrations ranging from 2.3 ng/mL in a controlled Karyun Jhuryun goat to a remarkably high 192 ng/mL in an uncontrolled sample from the same breed. These findings not only confirm the presence of oxytocin in goat and sheep milk but also demonstrate significant variability in its concentration.

The results highlight the reliability of the chromatographic method for milk samples. The data strongly suggest that oxytocin levels can fluctuate dramatically depending on breed and management practices. Given that the reported levels in uncontrolled samples (up to 192 ng/mL) far exceed the typical endogenous range (0.8–4.0 ng/mL) cited in the literature, this study supports the use of a threshold, such as the suggested 5.0 ng/mL, to help differentiate between natural and exogenous oxytocin administration.

Future research should focus on a more extensive investigation into the factors contributing to these variations, including environmental stressors, physiological states, and specific management practices. Further optimization of milk sample preparation to eliminate interfering compounds will also be crucial for enhancing the accuracy and robustness of future analyses.

#### CONFLICT OF INTEREST

Authors declare no conflict of interest

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#### AUTHOR CONTRIBUTION

Author 1, conceived the idea, did experimental work and prepared the initial draft of the paper,

while, Author 2, made proof reading and corrected minor mistakes.

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