Original Article

Comparative Study of Bacterial Contamination in Local Iraqi Sheep and Goats Semen

Mohammed Ansam Khalid

Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

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ABSTRACT

Background: Semen contamination is a detrimental factor in decreasing fertility. Seasonal changes may affect the contamination, too.

Objectives: This study was designed to detect semen contamination in ovine and caprine during different seasons.

Methods: Six fully mature male sheep and goats were subjected to electro-ejaculator collection twice monthly from February 1, 2022, to January 31, 2023 (Spring, February 1, 2022-April 30, 2022; Summer, May 1, 2022, July 31, 2022; Autumn August 1, 2022, October 31, 2022; Winter November 1, 2022, January 31, 2023), for studying the seasonal effect. A total of 288 semen samples were collected from both species (36 samples from each per season). All samples were subjected to bacterial isolation and identification.

Results: The results indicated that sheep semen had 4 different types of bacterial contamination with a higher number in contaminated samples than goat, which showed 5 different bacterial isolations. There were no significant differences (P>0.05) in bacterial contamination with regard to different seasons.

Conclusion: Different seasons appear not to affect bacterial contamination of semen in sheep and goats. Meanwhile, isolating all bacteria types was considered normal flora in both small ruminant species.

Keywords: Sheep, Goat, Semen, Bacterial contamination, Seasonal

*** Corresponding Author:**
Mohammed Ansam Khalid, PhD.
Address: *Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.*
Phone: *+096 (477) 19215359*
E-mail: *ansam@covm.uobaghdad.edu.iq*

Introduction

Microorganisms can affect male reproductive function either directly (reduce sperm motility, acrosomal reaction reduction, or increase sperm deformity) or indirectly (reactive oxygen species production) (Enwuru et al., 2016). However, diseases caused by them may reduce reproductive system immunity (Noakes et al., 2019). Also, mating with polluted semen is considered one of the etiologies of uterus infection (Pohjanvirta et al., 2020). So the male is responsible for semen pollution through contamination during semen collection with the glans penis and prepuce. Polluted semen negatively affects fertilization and acts as a genital disease carrier (Al-Zubaidy & Zaid, 2009). Semen contaminated with pathogens increases the threat of decreased fertility and reproduction effectiveness (Russell et al., 1997). Every semen ejaculate may contain contaminations of some nonpathogenic microbes that were not used in artificial insemination procedures. The massive accumulation of microbes leads to infertile mating (Thacker et al., 1984). Semen contamination with either pathogenic or nonpathogenic bacteria occurs during semen processing and storage. Then, these microbes will be transported along with the semen into the genital tract and cause severe diseases due to bacteremia or and viremia infections (Thibier & Guerin, 2000).

Many studies have identified bacterial contamination, such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, in the frozen semen of farm animals (Thibier & Guerin, 2000; Hobson et al., 2013). Semen that contains a variety of microorganisms reduces the survival rate and fertility of sperms, resulting in a decrease in offspring. Hence, reducing the contamination with microbes in semen is essential in the artificial insemination of the breeding stock (Schulze et al., 2020). Semen quality decreases when semen samples are contaminated with bacteria, fungi, and viruses; these pathogens are transmitted to the next generation (Mitra et al., 2016). Reproductive disorders may happen due to contamination with specific microbial agents during semen collection, handling, and preservation (Sannat et al., 2015). There is a correlation between bacterial load and semen quality, as an increase in bacterial numbers leads to a decline in sperm motility and viability (Reda et al., 2020). Meena et al., 2015 and Meena et al., 2017 reported that bulls frozen semen with higher microbial numbers showed a highly negative significant correlation ($P<0.01$) with progressive motility and vitality of sperms. Also, microbial toxins can indirectly affect sperm motility (Wang et al., 2021). Boar semen contaminated with bacteria was affiliated with a decline in sperm viability (Luther et al., 2023). Bacterial presence inside semen results in nutrient competition and metabolic byproduction that harm sperms (Luther et al., 2023). Furthermore, some dying bacteria damage the spermatozoa due to the release of lipopolysaccharides contained inside their walls. Bacterial inflammation of female genitalia may occur after being inseminated with infected semen (Morrell & Wallgren, 2014).

Thus, the bacterial contamination of semen causes adverse effects on sperm quality, either directly on the nutrients supply for sperms, which is represented in semen diluents, or indirectly by the formation of endotoxins and metabolic toxic byproducts (Fraczek & Kurpisz, 2015). Some studies have been conducted to study bacterial contamination (Hanoun & Al-Samrraae, 2019; Al-Taii & Yousif, 2019; Al-Taee et al., 2019; Razook et al., 2020; Gharban & Yousif, 2020; Foroutan et al., 2022; Gaddafi et al., 2023; Anvar et al., 2023) without dealing with semen samples. While other studies address the seasonal effect on genital organs without considering bacterial contaminations (Ibrahim & Zaid, 2015; Zaid, 2017; Ibahim & Zaid, 2017a; Ibahim & Zaid, 2017b). However, one study deals with the breeding season effect (Karasahin et al., 2023). This study was designed for the first time to investigate microbial contamination among sheep and goats in 4 consecutive seasons in Iraq.

Materials and Methods

Study animals

The study was conducted in a private property in Baghdad Province, Iraq, from February 1, 2022, to January 31, 2023. The animals were kept in ideal condition of management during the study. Semen was collected by electro-ejaculator every 15 days from 6 mature (3 years old) rams and bucks used for breeding during the study.

To study the seasonal effect, a total of 288 ejaculates were collected during the year:

1) Spring (February, March, April 2022) collection.

2) Summer (May, June, July 20222) collection.

3) Autumn (August, September, October 2022) collection.

4) Winter (November and December 2022 and January 2023) collection.

Bacterial identification and counting

Semen samples were cultured for bacterial detection and then incubated at 37°C in aerobic and anaerobic conditions for 24-48 hours. Bacterial isolation and identification were conducted following Quinn et al. (2006). The SAS software, version 2012, analysis system was undertaken to perform the chi-square test to detect the group variations.

Results

This study indicated that semen samples showed contamination with bacteria during the winter and summer seasons. In sheep, 6 positive isolations were recorded, representing 16.67% of 36 samples, while the spring and autumn seasons showed 5 positive samples or 13.89% of the total 36 semen samples. There were no significant variances (P>0.01) for samples of sheep semen between the seasons (Table 1). Table 2 shows goat semen samples. They revealed 6 positive samples during winter and summer, representing 16.67% of total samples, while in spring, it resulted in 5 positive samples (13.89%). Finally, autumn showed 3 positive samples (8.33%) of 36 tested samples. There was no significant difference (P>0.05) between all seasons (Table 2). The total percentage of positive samples for all the seasons in sheep and goats shows that the sheep had more contaminated semen (22 [15.28%] of 144) samples than the goats (20 [13.89%] of 144). There were no significant differences (P>0.05) between sheep and goats regarding total semen contaminations (Tables 1 and 2). In contaminated sheep semen, isolated bacteria types were identified as *Klebsiella pneumonia*, *Proteus mirabili*s, *E. coli*, and *S. aureus* (Table 3). In contrast, in goat semen samples, we found *S. aureus*, *Streptococcus faecalis, P. aeruginosa*, *E. coli*, and *P. mirabilis* (Table 4). The single isolation and mixed contaminated samples from sheep were 9 and 13, respectively; the single isolation and mixed contaminated samples from goats were 12 single and 8, respectively (Tables 3 and 4).

Discussion

This study is the first to investigate semen contamination in 4 consecutive seasons. Results indicated that 15.28% of sheep and 13.89% of goats semen samples were contaminated. Also, sheep's semen was contaminated with 4 different bacterial types compared to 5 different bacterial isolates in goats' semen. There was no

Table 1. The number of bacterial isolation samples, positive results, and percentage during different seasons of sheep ram semen samples

Season	Sample No.	Positive Isolation	%	P
Winter	36	6	16.67	
Spring	36	5	13.89	
Summer	36	6	16.67	0.973*
Autumn	36	5	13.89	
Total	144	22	15.28	

*Not significant (P>0.05).

Table 2. The number of bacterial isolation samples, positive isolation, and percentage during different seasons of goat buck semen samples

Season	Samples No.	Positive Isolation	%	P
Winter	36	6	16.67	
Spring	36	5	13.89	
Summer	36	6	16.67	0.503*
Autumn	36	3	8.33	
Total	144	20	13.89	

*Not significant (P>0.05).

Table 3. Types of isolated bacteria from semen during different seasons in sheep ram

Season	Bacterial Isolated Types	Single or Mixed Isolation
Winter	*K. pneumonia*	3 singles
	P. mirabilis	3 mixed
Spring	*K. pneumonia*	2 singles
	P. mirabilis	3 mixed
Summer	*E. coli*	2 singles
	S. aureus	4 mixed
Autumn	*E. coli*	2 singles
	S. aureus	3 mixed
Total	*K. pneumonia* *P. mirabilis* *E. coli* *S. aureus*	9 singles 13 mixed

method to determine how an ejaculate be contaminated with bacteria (Azawi & Ismaeel, 2011). The bacterial types that are mostly isolated are *S. aureus, Enterobacter cloacae*, *P. mirabilis*, *Staphylococcus epidermis*, and *E. coli*. These 5 bacterial types represent 97% of all contaminated samples, as Yaniz et al. (2010) mentioned, which agrees with this study regarding sheep and partially regarding goat's contaminated semen. During storage, samples with less than 100 colony-forming units (CFU)/mL enterobacteria count had faster progressive sperm motility with higher velocity at different times (Yaniz et al., 2010). The contaminated semen with *E. coli* during storage showed a downgrade in viability, motility, and speed (Yaniz et al., 2010). Sheep's semen

Table 4. Types of isolated bacteria from semen during different seasons in goat buck

Season	Bacterial Isolated Types	Single or Mixed Isolation
Winter	*E. coli*	4 singles
	P. aeruginosa	2 mixed
Spring	*E. coli*	2 singles
	S. aureus	3 mixed
Summer	*S. faecalis*	3 singles
	S. aureus	3 mixed
Autumn	*P. mirabilis*	3 singles
Total	*E. coli* *P. aeruginosa* *S. faecalis* *S. aureus* *P. mirabilis*	12 singles 8 mixed

contaminated with enterobacterial species seemed low in spermatozoa quality during cooling at 15°C (Yaniz et al., 2010). This risk appears possible as our study samples became contaminated with such bacterial species. There was a statistical decrease ($P<0.01$) in the mean number of live bacteria during the middle of winter extended into spring ending; January showed lower bacterial count (60.5±2.98) with season significant effect in Awassi ram semen on bacterial number (Azawi & Ismaeel, 2011). This finding agrees partially with this study's results. There is no consensus over the bacterial role within semen, so it is difficult to determine the cause of such contamination. Bacterial presence in semen indicates infection, which is proved by transmitting this infection to the genital organs (Sanocka-Maciejewska et al., 2005). Sperm morphology was affected by inflammatory cells in some cases of bacterial contamination. Also, bacterial presence in semen ejaculates directly affects fertilization of ova (Fraczek & Kurpisz, 2015) by attaching with spermatozoa, decreasing their motility and inducing acrosome reaction (Azawi & Ismaeel, 2011). Microbes also indirectly affect through toxins production (Wang et al., 2021). Semen has good quality when it contains low bacterial contamination (Azawi & Ismaeel, 2011). Semen viability decreases within a short time if their sperms are contaminated, resulting in sperm death and increasing the risk of female reproductive tract pathologies and, eventually, decreasing fertility (Azawi & Ismaeel, 2011).

This fact shows the importance of detecting any bacterial contamination of semen before using it in breeding. Some bacterial types are presented in the genital system of the females without any effect on the function of reproduction. Zaid (2009) determined the bacterial microflora isolation inside the ovine vagina between 56.5%-96.5%, while it was 71.4% in the caprine reported by Al-Delemi (2005) during the diestrus phase. Another factor can be the contamination of male external genital parts during mating. Zaid (2009) identified more than one bacterium in one swab. This finding is similar to our results. In the ewe, a percentage of large bacteria count had been isolated as *E. coli* and *Enterobacter* species with some other bacteria. This finding agrees with other research results (Al-Delemi, 2005). Otherwise, the does had the highest percentage of isolates with *S. faecalis* and *P. aeruginosa* (Zaid, 2009), which agrees with this study. This study revealed that the external parts of the genital system of rams carried many bacterial species. This finding agrees with other studies (Al-Zubaidy & Zaid, 2009; Pohjanvirta et al., 2020). The present results revealed that the bacteria cultured from male semen were partly similar to those found in other studies (Zaid et al., 2007). A normal microflora contains many bacteria that can proliferate, decrease body immunity, and cause diseases, while others are pathogenic (Al-Zubaidy & Zaid, 2009). The viability of sperm fertilization has an important relationship with bacterial number and bacterial types (Al-Zubaidy & Zaid, 2009). However, Al-Zubaidy and Zaid, 2009 found a 10-fold bacterial count in the second ejaculation compared to the first. This result may explain the decrease in the contamination percentage of goats during the breeding season in our research. The present study showed that more than one type of bacteria can be cultured from the same swab, which is consistent with other studies (Al-Delemi, 2005). Normal male semen contains Staphylococcus aureus in large amount (Al-Zubaidy & Zaid, 2009). This finding agrees with our study. After mating, *P. aeruginosa* was isolated by Al-Delemi (2005), which was considered normal flora in ewes. In this study, such bacterium was detected in male goats. Semen bacterium relates to low fertility and male sterility (Al-Zubaidy & Zaid, 2009). The contamination of semen with *E. coli* or *P. aeruginosa* may result from feces during male mating (Al-Zubaidy & Zaid, 2009). There was a seasonal effect on semen quality in different animal species (Ibrahim & Zaid, 2015; Zaid, 2017; Ibahim & Zaid, 2017a; Ibahim & Zaid, 2017b). In this part, these studies did not deal with bacterial contamination like we did in our research.

Conclusion

The semen of sheep and goats had been contaminated with normal flora bacteria out of breeding uses, while the seasons did not affect this bacterial contamination.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Conflict of interest

The author declared no conflict of interest.

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