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Comparative Study of Bacterial Contamination between Local Iraqi Sheep and Goat Semen

Ansam Khalid Mohammed

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

Abstracts

Background: Semen contamination is a key factor in decreasing fertility and the seasonal effect may act in this part.

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

Methods: Six fully mature male of both sheep and goat were subjected to electro-ejaculator collection twice monthly for the period 1/2/2022 to 31/1/2023, (Spring, 1/2/2022-30/4/2022,

Summer,1/5/2022-31/7/2022, Autumn 1/8/2022-31/10/2022, Winter 1/11/2022-31/1/2023), for studying the seasonal effect. A total of 288 semen samples were collected from both species (36 samples from each per season). Bacterial isolation and identification applied to all samples. **Results:** The results indicated that sheep semen had four different types of bacterial contamination with higher number in contaminated samples than goat which showed five different bacterial isolation, with no significant differences (P>0.05) in bacterial contamination under effect of different seasons. The result of the herein study indicated that the sheep had four different types of contaminated bacteria with higher contamination number than goat, while the goat show five different bacterial isolation. There was no significant differences (P>0.05) in bacterial contamination under effect of different seasons.

Conclusions: from this we conclude that the different seasons appears to have no effect on bacterial contamination of semen in sheep and goat, meanwhile the isolation of all bacteria types was considered as normal flora in both small ruminants' species.

Keyword: sheep, goat, semen, bacterial contamination, seasonal.

Introduction

Microorganisms can affect male reproductive function either directly (reduce sperm motility, acrosomal reaction reduction or increase sperm deformity) or indirectly (reactive oxygen production) (Enwuru *et al*., 2016). However, disease caused by them may reduce reproductive system immunity (Noakes *et al*., 2019). Also, mating, or polluted semen, is considered one of the factors of uterus infection (Pohjanvirta *et al*., 2020), so the male is responsible for semen pollution through contamination during semen collection with glans penis and prepuce (Zaid and Al-Zubaidy, 2009). Polluted semen negatively affects fertilization and acts as a genital disease carrier (Zaid and Al-Zubaidy, 2009). Semen contaminated with pathogens increases the threat to decrease fertility and reproduction effectiveness (Russell *et al*., 1997). Every semen ejaculates may contain contaminations of some nonpathogenic microbes which were not used in artificial insemination procedures. The massive gathering of microbes leads in infertile mating (Thacker *et al*., 1984). Semen contamination with either pathogenic or non-pathogenic bacteria occur during semen processing and semen storage, then these microbes will be transported through the semen into genital tract and cause serious diseases due to bacteremia or/and viraemia infections (Thibier and Guerin, 2000)**.** Many studies were identifying bacterial contamination in the frozen semen of farm animals such as *E. coli*, *Staph. aureus*, and *Pseudo. aeruginosa* (Thibier and Guerin, 2000; and Hobson *et al*., 2013). Semen that contains variety of microorganisms reduces the survival rate and fertility of sperms; resulting decrease in offspring, so that the suppression contamination with microbes in semen is essential in artificial insemination of the breeding stock (Schulze *et al*., 2020). Semen quality decreased when semen samples are contaminated with bacteria, fungi and viruses, these pathogens transmitted to next generation (Mitra *et al*., 2016). Reproductive disorder may happen due to contamination with specific microbal agents during semen collection, handling and preservation (Sannat *et al*., 2015). There is a correlation between bacterial load and semen quality as it had had been shown that the increase of bacterial numbers led to declivity in sperm motility and viability (Reda *et al*., 2020). Meena *et al*., (2015 and 2017),

Reported that bulls frozen semen with higher microbial numbers are of highly negative significant correlation ($P<0.01$) with progressive motility and, vitality of sperms. Also, microbial toxins can indirectly affect sperms 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 nutrients competition and metabolic byproduction that harm sperms (Luther *et al*., 2023), in addition to all of that, some bacteria, when they die, cause damage to 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 and Wallgren, 2014). As above the bacterial contamination of semen cause adverse effect on sperm quality, either directly, on nutrients supply for sperms which is represented in semen diluents, or indirectly by formation of endotoxins and metabolic toxic byproducts (Fraczek and Kurpisz, 2015). Some of studies were conducted in order to study the bacterial contamination (Hanoun and Al-Samrraae, 2019; Al-Taii and Yousif, 2019, Al-Taee *et al*., 2019; Razook *et al*., 2020; Gharban and Yousif, 2020; Foroutan *et al*., 2022; Gaddafi *et al*., 2023 and Anvar *et al*., 2023) without dealing with semen samples. While other studies address the season effect on genital organs without refereeing to bacterial contaminations (Ibrahim and Zaid, 2015; Zaid, 2017; Ibahim and Zaid, 2017a; 2017b), one study deals with breeding season effect (Karasahin *et al*., 2023). This study was designed to investigate the microbial contamination types among sheep and goat in 4 consecutive seasons in Iraq for the first time.

Material and Methods

Animals: The study was conducted in a private property in Baghdad province from 1/2/2022 to 31/1/2023. Animals were kept in ideal condition of management during the course of the study. Semen collected by electro-ejaculator every 15 days from six mature (3 years old) rams and bucks that were used for breeding during the course of the study.

To study the seasonal effect, a total collection of 288 ejaculates along the year were spread into:

1) Spring season (2/2022, 3/2022 and 4/2022) collection.
2) Summer season (5/2022, 6/2022 and 7/2022) collection.
3) Autumn season (8/2022, 9/2022 and 10/2022) collection.
4) Winter (11/2022, 12/2022 and 1/2023) collection.

Bacterial identification and counting: Semen samples were cultured for bacterial detection and then incubated at 37 ºC in aerobic and anaerobic conditions for 24-48 hours. Bacterial isolation and identification were conducted following Quinn *et al*. (2006). SAS (2012) analysis system was conducted to perform Chi-square test to detect the variation between the groups.

Results

This study indicated that semen samples showed contamination with bacteria during winter and summer seasons. In sheep, six positive isolations recorded, which represent (16.67%) of 36

samples, while the spring and autumn seasons showed five positive samples (13.89%) from total 36 semen samples (Table 1). No significant variances (P>0.01) for samples of sheep semen between among seasons (Table 1). Table (2) shows that goat semen samples had six positives during winter and summer which represent (16.67%) of total samples, while in spring appear to result five positive samples (13.89%). Finally, autumn showed 3 positive samples (8.33%) of 36 tested samples. There was no significant differences (P>0.05) between all seasons (Table 2). Total percentage of positive samples for all the seasons in sheep and goat show that the sheep had more contaminated semen 22 of 144 (15.28%) samples as comparative with goat 20 of 144 (13.89%) (Table 1 and 2). There were no significant differences (P>0.05) between total semen contaminations between sheep and goat (Table 1 and 2). In contaminated sheep semen, isolated bacteria types identified are: *Klebsiella pneumonia*, *Proteus mirabilis*, *Escherichia coli* and *Staphylococcus aureus* (Table 3), whereas in goat semen goat are: *Staphylococcus aureus*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *E. coli* and *Proteus mirabilis* (Table 4). The single isolation samples from sheep were 9 with 13 mixed contaminated samples, as well as 12 single and 8 mixed contaminated goat semen (Table 3 and 4).

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

Seasons	Samples number	Positive isolation	Percentage %	P value
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%	

* insignificant (P>0.05).

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

Seasons	Samples number	Positive isolation	Percentage %	P value
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%	

* insignificant (P>0.05).

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

Seasons	Bacterial isolated types	Single or mixed isolation
Winter	*Klebsiella pneumonia*	3 single
	Proteus mirabilis	3 mixed
Spring	*Klebsiella pneumonia*	2 single
	Proteus mirabilis	3 mixed
Summer	*Escherichia coli*	2 single
	Staphylococcus aureus	4 mixed
Autumn	*Escherichia coli*	2 single
	Staphylococcus aureus	3 mixed

Total	*Klebsiella pneumonia* *Proteus mirabilis* *Escherichia coli* *Staphylococcus aureus*	9 single 13 mixed

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

Seasons	Bacterial isolated types	Single or mixed isolation
Winter	*Escherichia coli* *Pseudomonas aeruginosa*	4 single 2 mixed
Spring	*Escherichia coli* *Staphylococcus aureus*	2 single 3 mixed
Summer	*Streptococcus faecalis* *Staphylococcus aureus*	3 single 3 mixed
Autumn	*Proteus mirabilis*	3 single
Total	*Escherichia coli* *Pseudomonas aeruginosa* *Streptococcus faecalis* *Staphylococcus aureus* *Proteus mirabilis*	12 single 8 mixed

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Discussion

This study is the first trial to investigate the semen contamination in four consecutive seasons. Results indicated that sheep semen are contaminated with 15.28% while in goat's its 13.89%, but sheep's semen are contaminated with four different bacterial types compared to five different bacterial isolates in goat semen. There was no perfect ability to determine the way of the bacterial contamination in an ejaculate (Azawi and Ismaeel, 2011). Bacteria types that are mostly isolated were *Staphylococcus aureus*, *Enterobacter cloacae*, *Proteus mirabilis*, *Staphylococcus epidermis*, and *Escherichia coli*, species. These five bacterial types represent 97% of all contaminated samples as mentioned by Yaniz *et al.* (2010), which agree with this study in regard to sheep and partially in regard to 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). *E. coli* contaminated semen during storage showed downgrade in their viability, motility and velocity (Yaniz *et al*., 2010). Sheep's semen with enterobacterial species contamination seemed lower in spermatozoa quality during cooling at 15°C (Yaniz *et al*., 2010). This risk becomes true as the samples of our study became contaminated with such bacterial species. There was a statistical decrease ($P<0.01$) in the mean of number of live bacteria during middle of winter extended into spring ending, January showed lower of bacterial count (60.5±2.98) with season significant effect in Awassi ram semen on bacterial number (Azawi and Ismaeel, 2011). This agrees partially with this study results. Bacterial presence role within semen has a wide disagreement opinion, so it is difficult to determine the cause of such contamination. Bacterial presence with semen, surly indicates infection, due to transmission of this infection to the genital organs which can occur (Sanocka-Maciejewska *et al*., 2005). Sperm morphology was affected with inflammatory cells in some cases of bacterial contamination, and bacterial presence in semen ejaculates affected directly on

fertilization of ova (Fraczekand Kurpisz, 2015), by attaching with spermatozoa, decreasing their motility and inducing acrosome reaction (Azawi and Ismaeel, 2011). Microbes affect indirectly through toxins production (Wang *et al*., 2021). Semen is considered as a good quality when there is low bacterial contains (Azawi and Ismaeel, 2011). Semen viability decreases within a short time if their sperms are contaminated, resulting sperms death leading to the risk of increasing female reproductive tract pathologies, and eventually, decreasing fertility (Azawi and Ismaeel, 2011). This fact shows the importance of detecting any bacterial contamination of semen before using it in breeding. Some bacterial types presented in the genital system of the females without any effect upon the function of reproduction (Zaid, 2009). Zaid (2009) determine the bacterial microflora isolation inside ovine vagina between 56.5-96.5%, while in the caprine (71.4%) has been reported by Al-Delemi (2005) during diestrous phase. Another factor can be the male external genital parts contamination during matting with female. The isolation of more than one bacterium in one swabs was identify by Zaid (2009). This fact is similar to our results. In the ewe percentage of large bacteria count which had been isolated as *Escherichia coli* and Enterobacter spices with some other bacteria. This agrees with other researchers results found before (Al-Delemi, 2005). Otherwise, the does had highest percentage isolates with *Streptococcus faecalis* and *Pseudomonas aeruginosa* (Zaid, 2009) which agrees with this study. This study revealed that the external parts of genital system of rams were carrying many bacterial species. This finding agrees with other authors (Zaid and Al-Zubaidy, 2009 and Pohjanvirta *et al*., 2020). The resent results revealed that the bacteria which cultured from male semen were partly similar to other authors (Zaid *et al*., 2007). A normal microflora contains many bacterial types can grow rapidly and decrease body immunity causing diseases, while there are other which are considered as pathogenic types (Zaid and Al-Zubaidy, 2009). Viability of sperm fertilization with bacterial number and bacterial types is an important relationship (Zaid and Al-Zubaidy, 2009). However Zaid and Al-Zubaidy (2009) found that there were ten folds little bacterial count in the second

ejaculation compared to the first. This result may be explaining the decrease of contamination percentage during breeding season of goats in our research. The recent study showed that it can be culture more than one type of bacteria from the same swab; this fits with other authors (Al-Delemi, 2005). Normal male semen contains *Staphylococcus aureus* with large amount (Zaid and Al-Zubaidy, 2009).This agrees with our study. After mating *Pseudomonus aeruginosa* isolated by (Al-Delemi, 2005) and it is considered as normal flora in ewes. In this study the isolation of such bacterium is done in male goats. Semen bacterium refers to low fertility; also, there was a relationship with male sterility (Zaid and Al-Zubaidy, 2009). The contamination of semen with *Esherichia coli* or *Pseudomonus aeruginosa* may be resulted from feces presence during mating of the male (Zaid and Al-Zubaidy, 2009). There was a seasonal effect on semen quality in different animal species (Ibrahim and Zaid, 2015; Zaid, 2017; Ibahim and Zaid, 2017a; 2017b). In this part these studies did not deal with bacterial contamination as that which done by our study.

Conclusion

the semen of sheep and goat had contaminated with normal flora bacteria out of breeding uses, while the seasons had no effect on this bacterial contamination.

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