

The influence of semen collection frequency on the deleterious interaction between the enzymes of the bulbourethral gland and egg yolk during the dilution of buck semen

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Abstract

The study aimed to evaluate the influence of semen collection frequency at different protocols on deleterious the interaction between the enzymes of the bulbourethral gland and egg yolk during the dilution and of buck semen. Five bucks aged 2.5-3 years were included in this study. Four different protocols of semen collection frequency were applied; once a week, twice a week at an interval of two days, thrice a week at an interval of one day, and twice daily at an interval of one hour. Buck semen ejaculates were evaluated using computer assisted sperm analysis that includes the percentage of motile sperm (MS), percentage of progressive motile sperm (PMS), velocity curvilinear (VCL), velocity average path (VAP), velocity straight line (VSL), linearity (LIN), straightness (STR), wobble (WOB), amplitude of lateral head movement (ALH), and beat cross frequency (BCF). Results showed that sperm motility parameters had decreased significantly after dilution. Sperm motility parameters (VCL, VSL, VAP and ALH) of the second ejaculate were higher significantly ($P<0.05$) after dilution, when ejaculates collected twice and thrice a week and twice a day. Conversely, motility parameters of third ejaculates were less than those of first and second ejaculates when ejaculates collected thrice a week. In conclusion, there are effects of semen collection frequency on the ability of buck semen dilution, and the best results were when ejaculates had collected twice a day at an interval of one hour, accordingly we recommended this protocol for preparation of buck semen for artificial insemination.

Keywords: Bulbourethral gland, Egg yolk, Dilution, Buck, Semen.

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INTRODUCTION

Artificial insemination has an important role in the improvement of the goat production (milk, hair, and meat) (Leboeuf et al., 2000). Successive artificial insemination depends mainly on the semen collection, dilution and preservation. Currently, the semen extenders containing egg yolk and milk widely used for dilution of small ruminant semen (Salamon and Maxwell, 2000).

The process of semen storage (cold or frozen) causes damage in the composition and function of spermatozoa, leading to a decline in the motility, viability and fertility of sperm, so it was a goal of many studies reducing or resolving this problem. Dilution and preservation of goat semen has a special problem, it is the adverse effect of seminal plasma on the sperm viability when semen diluted with an extender containing egg yolk or milk (Leboeuf et al., 2000).

The main problem of the goat seminal plasma comes from the secretion of the bulbourethral gland which containing a phospholipase A that called egg yolk coagulating enzyme (Roy, 1957) and glycoprotein-60 (Pellicer-Rubio et al. 1997).

Egg yolk coagulating enzyme hydrolyzing the egg yolk phospholipids to produce lysophospholipids, the main type of these lysophospholipids is lysolecithins which have a toxic effect on spermatozoa (Aamdal et al., 1965). It was also shown that an adverse effect of egg yolk coagulating enzyme by hydrolysis of the phosphatidycholine in egg yolk to fatty acids and lysophosphatidycholine. Production of fatty acids leading to a decrease in the pH of diluted semen, which causes a reduction in sperm viability (Iritani and Nishikawa, 1972), while production of lysophosphatidycholine has a toxic effect on

sperm motility and sperm cell membrane integrity, which leads to reduce the sperm fertility (Upreti et al., 1999).

The adverse effect produced by glycoprotein-60 coming from the triacylglycerol hydrolase activity of glycoprotein-60 which causing disruption of the sperm cell membrane and decreasing sperm motility (Pellicer-Rubio et al. 1997).

Several studies were conducted to reduce the adverse effect of bulbourethral gland secretion which includes; removal of seminal plasma (Naing et al., 2011; Santiago-Moreno et al., 2017), washing of spermatozoa (Cabrera et al., 2005; Salvador et al., 2007), using different concentrations of egg yolk (Priyadharsini et al., 2001; Dhaher and Aziz, 2013), adding egg yolks of different species to extender (Swelum et al., 2018), replacement of egg yolk by bovine serum albumin (Azawi and Salmanand, 2014), collection of semen by different methods (Jiménez-Rabadána et al., 2012; Dhaher and Aziz, 2013), dilution of buck semen at different rates (Khalifa et al., 2006), and dialysis of the goat semen (Bajuk et al., 2018).

The present study aimed to evaluate the influence of semen collection frequency at different protocols on deleterious the interaction between the enzymes of the bulbourethral gland and egg yolk during the dilution and of buck semen.

MATERIALS AND METHODS

Animals

The study was carried out in the Artificial Insemination Laboratory of the College of Veterinary Medicine, University of Mosul. Five mature Iraqi black bucks aged 2.5-3 years and weighing 48-57 kg were used in this study. All bucks were examined clinically, they were healthy and free from any diseases or obvious

abnormalities of the reproductive organs and they have good libido. The bucks were housed under the same management and nutritional condition. Two weeks before starting of the study, the bucks were trained for artificial vagina semen collection.

Semen collection

An artificial vagina was used for collection of buck semen. The artificial vagina was prepared by filling it with warm water (39-40 °C) and injected of air to provide the temperature and pressure that required for ejaculation. The latex inner liner of the artificial vagina was lubricated by vaseline. The semen was collected into graduated test tube that fixed to the narrow end of the collecting cone when the buck mounted a doe in heat.

Four different protocols of semen collection frequency were applied; first protocol, semen samples were collected once a week for a period of three weeks, second protocol, ejaculates were collected twice a week at an interval of two days for a period of two weeks, third protocol, semen samples were collected thrice a week at an interval of one day for a period of two weeks, fourth protocol, ejaculated were collected twice daily at an interval of one hour for a period of two weeks. The animals were given a rest for one week between the applied protocols to prevent the interaction between the protocols and to give an appropriate time for the bulbourethral gland to return to normal function and secretion.

Semen dilution

Egg yolk citrate extender was used for dilution of buck ejaculates. Citrate buffer was prepared by dissolve of 2.9 g sodium citrate, 1.3 g D-glucose, 0.068 g penicillin-G and 0.1 g streptomycin sulphate in 100 ml of distilled water. According to our

previous study (Dhaher and Aziz, 2013) egg yolk was added to buffer at a ratio of 10 %. Immediately after collection, semen samples were extended in the diluent to a final concentration of 400×10^6 motile spermatozoa/ml.

Semen analysis

The computer assisted sperm analysis (microptic s.l., Barcelona, Spain) and Sperm Class Analyzer® (SCA®, v.4.2) were used for evaluation of the buck semen ejaculates. The following sperm motility parameters were evaluated; percentage of motile sperm (MS) (%), percentage of progressive motile sperm (PMS) (%), the values of the velocity curvilinear (VCL) (microns/sec), velocity average path (VAP) (microns/sec), velocity straight line (VSL) (microns/sec), linearity (LIN) (%), straightness (STR) (%), wobble (WOB) (%), amplitude of lateral head movement (ALH) (microns/sec), and beat cross frequency (BCF) (Hz). Each ejaculate was evaluated three times, and for each evaluation ten fields were examined that included at least a total of three hundred sperms (Kozdrowski et al. 2007).

Statistical Analyses

Data of sperm motility parameters of ejaculates that collected in the four different protocols of semen collection frequency were presented as mean \pm standard error (SE). The significant differences between the tested parameters were determined by t-test and one-way analysis of variance (followed by Duncan's multiple range test). Statistical analyses were carried out using Sigma Stat (Jandel scientific software V3.1). $P < 0.05$ was considered as statistically significant.

RESULTS

Table 1 presents the sperm motility parameters of raw buck ejaculates prior to dilution and the changes in these parameters after dilution of semen with a diluent containing 10% egg yolk. It was observed that there was no significant decrease in MS and PMS percentages after dilution. Other sperm motility parameters of ejaculates except BCF have been decreased significantly ($P<0.05$) after dilution compared to those values that recorded before dilution.

Table 1: Semen and sperm motility parameters of bucks prior and after dilution with a diluent containing 10% egg yolk

Semen and sperm motility parameters	Prior dilution	After dilution
Volume (ml)	1.14 ± 0.11	-----
Concentration (x 10 ⁹)	2.07 ± 0.23	-----
SM (%)	84.9 ± 5.4	83.3 ± 4.2 ^{NS}
PSM (%)	27.4 ± 3.2	22.7 ± 2.6 ^{NS}
VCL (microns/sec)	84.1 ± 0.30	72.3 ± 3.2 ^{**}
VSL (microns/sec)	48.8 ± 0.19	43.5 ± 0.13 ^{***}
VAP (microns/sec)	69.6 ± 0.21	57.6 ± 0.21 ^{***}
LIN (%)	54.1 ± 0.19	49.0 ± 0.23 ^{**}
STR (%)	64.7 ± 0.22	60.0 ± 0.26 [*]
WOB (%)	68.8 ± 0.13	61.3 ± 0.17 ^{**}
ALH (microns/sec)	3.8 ± 0.1	2.9 ± 0.1 ^{***}
BCF (Hz)	5.8 ± 0.3	6.2 ± 0.3 ^{NS}

^{NS} not significant, ^{*} significant decrease ($P<0.05$), ^{**} significant decrease ($P<0.01$), ^{***} significant decrease ($P<0.001$).

Table 2 summarizes the results of buck sperm motility parameters of the first and

second ejaculates that collected in the second protocol of this study (twice a week collection for a period of two weeks) after dilution with a diluent containing 10% egg yolk. The most sperm motility parameters that include MS, PMS, LIN, STR, WOB, ALH and BCF of the second ejaculates were more than those of the first ejaculates. The remaining sperm motility parameters that include VCL, VSL and VAP of the second ejaculates were higher significantly ($P<0.001$) than those parameters of the first ejaculate.

Table 2: Comparison of buck semen and sperm motility parameters between the first and second ejaculates that collected in protocol of twice a week collection for a period of two weeks after dilution with a diluent containing 10% egg yolk (n = 10, Mean ± SE)

Semen and sperm motility parameters	First ejaculate	Second ejaculate
Volume (ml)	1.16 ± 0.11	1.06 ± 0.14 ^{NS}
Concentration (x 10 ⁹)	2.23 ± 0.21	1.92 ± 0.22 ^{NS}
SM (%)	81.75 ± 3.7	91.06 ± 6.4 ^{NS}
PSM (%)	30.06 ± 2.4	31.73 ± 2.1 ^{NS}
VCL (microns/sec)	82.87 ± 0.71	89.44 ± 0.93 [*]
VSL (microns/sec)	44.95 ± 0.52	50.37 ± 0.92 [*]
VAP (microns/sec)	60.86 ± 0.54	67.67 ± 0.92 [*]
LIN (%)	55.16 ± 0.49	54.66 ± 0.68 ^{NS}
STR (%)	70.36 ± 0.46	71.15 ± 0.68 ^{NS}
WOB (%)	73.54 ± 0.32	74.20 ± 0.41 ^{NS}
ALH (microns/sec)	2.92 ± 0.29	3.11 ± 0.35 ^{NS}
BCF (Hz)	7.14 ± 0.6	7.56 ± 0.84 ^{NS}

^{NS} not significant, ^{*} significant variation ($P<0.001$).

Results of the third protocol of semen collection in this study which were buck ejaculates collected thrice a week for a

period of two weeks are presented in table 3.

Table 3: Semen and sperm motility parameters of bucks that collected in protocol of thrice a week for a period of two weeks after dilution with a diluent containing 10% egg yolk (n = 10, Mean \pm SE)

Semen and sperm motility parameters	First ejaculate	Second ejaculate	Third ejaculate
Volume (ml)	1.21 \pm 0.15 ^a	1.17 \pm 0.17 ^a	0.98 \pm 0.12 ^a
Concentration (x 10 ⁹)	2.08 \pm 0.27 ^a	2.20 \pm 0.23 ^a	2.12 \pm 0.21 ^a
SM (%)	89.26 \pm 4.20 ^a	92.86 \pm 3.30 ^a	79.79 \pm 5.10 ^a
PSM (%)	25.67 \pm 1.90 ^a	29.51 \pm 1.70 ^a	26.41 \pm 2.20 ^a
VCL (microns/sec)	77.74 \pm 0.6 ^b	81.85 \pm 0.72 ^a	69.27 \pm 0.53 ^c
VSL (microns/sec)	43.35 \pm 0.54 ^b	45.34 \pm 0.53 ^a	39.68 \pm 0.46 ^c
VAP (microns/sec)	54.34 \pm 0.56 ^b	61.46 \pm 0.61 ^a	53.89 \pm 0.49 ^b
LIN (%)	51.86 \pm 0.36 ^a	52.31 \pm 0.38 ^a	51.75 \pm 0.35 ^a
STR (%)	66.37 \pm 0.34 ^a	65.58 \pm 0.37 ^a	65.84 \pm 0.33 ^a
WOB (%)	71.64 \pm 0.25 ^a	72.11 \pm 0.36 ^a	70.88 \pm 0.23 ^a
ALH (microns/sec)	2.44 \pm 0.11 ^b	2.88 \pm 0.14 ^a	2.37 \pm 0.13 ^b

^{a,b,c} different small letters in each row refer to significant variation ($P < 0.05$).

All sperm motility parameters (after dilution with a diluent containing 10% egg yolk) of the second ejaculates were higher in comparison with first ejaculates, the significant ($P < 0.05$) variation were recorded only in four sperm parameters that included VCL, VSL, VAP, and ALH. In contrast to the results of second ejaculates, the sperm motility parameters of third ejaculates were less than those of first and second ejaculates, especially the VCL, VSL, VAP and ALH which were lower significantly ($P < 0.05$) if they were

compared with their values in second ejaculates.

Comparison between the results of sperm motility parameters of the first and second ejaculates that were collected in the fourth protocol of semen collection (twice daily, at one day each week) are summarized in table 4.

Table 4: Results of semen and sperm motility parameters of bucks that collected in protocol of twice daily at one day each week for a period of two weeks after dilution with a diluent containing 10% egg yolk (n = 10, Mean \pm SE)

Semen and sperm motility parameters	First ejaculate	Second ejaculate
Volume (ml)	1.04 \pm 0.13	0.98 \pm 0.14 ^{NS}
Concentration (x 10 ⁹)	2.28 \pm 0.61	2.09 \pm 0.52 ^{NS}
SM (%)	82.52 \pm 4.63	95.30 \pm 3.93 [*]
PSM (%)	34.68 \pm 2.16	39.77 \pm 1.01 [*]
VCL (microns/sec)	71.77 \pm 0.54	75.34 \pm 0.76 [*]
VSL (microns/sec)	39.91 \pm 0.47	49.43 \pm 0.71 ^{***}
VAP (microns/sec)	54.56 \pm 0.49	60.17 \pm 0.73 ^{**}
LIN (%)	51 \pm 0.42	63 \pm 0.54 ^{***}
STR (%)	66 \pm 0.43	73 \pm 0.51 ^{***}
WOB (%)	73 \pm 0.24	80 \pm 0.34 ^{***}

^{NS} not significant, ^{*} significant variation ($P < 0.05$), ^{**} significant variation ($P < 0.01$), ^{***} significant variation ($P < 0.001$).

After dilution of the buck ejaculates with a diluent containing 10% egg yolk, all sperm motility parameters of the second ejaculate were higher than those of the first ejaculate. The highest significant ($P < 0.001$) variations were observed in VSL, LIN, STR and WOB, significant ($P < 0.01$) variation was observed in VAP, other parameters of

the second ejaculates that included SM, PSM, VCL, and ALH were varied significantly ($P < 0.05$) than those of first ejaculates, while there was no significant variation in BCF between the two ejaculates.

DISCUSSION

The effect of semen collection frequency on the semen quality has already studied in farm animals; such as bucks (Oyeyemi et al., 2000), rams (Aguirre et al., 2007; Yotov et al., 2011), boars (Pruneda et al., 2005), bulls (Rashid et al., 2015) and camels (Al-Bulushi et al., 2018), these studies have limited to evaluate the effect of semen collection frequency on the semen characteristic. Our study has been planned to observe the effect of semen collection frequency on the success of the buck semen dilution. There were two main reasons for this study, the first one was that the references indicate that the quality of semen has changed by the repetition of the semen collection, these changes include the volume of ejaculates, semen concentration and percentage of motile sperms (Ritar et al., 1992; Oyeyemi et al., 2000). The second reason is our suggestion that the frequent semen collection may be having a role in reducing the amount of seminal plasma, consequently that can be reducing the amount of bulbourethral gland enzymes that have an interaction with egg yolk during the buck semen dilution.

Notwithstanding we diluted the semen at low concentration of egg yolk (10%), nevertheless a significant reduction in sperm motility parameters was observed after dilution of buck ejaculates. This can be attributed to the harmful interaction between buck seminal plasma content and egg yolk (Leboeuf et al., 2000), same

significant differences assessed between fresh and frozen thawed samples (Dorado et al., 2010).

Whether the ejaculates were collected twice or thrice a week, or twice a day the sperm motility parameters of the second ejaculates, especially the values of VCL, VSL, VAP, and ALH, were higher significantly than those of first ejaculates, notwithstanding there were no significant differences in the percentage of MS and PMS between the two ejaculates. The best results of sperm motility parameters of the second ejaculates that obtained in the current study agreed with results of Yotov et al., (2011) which attributed the improvement of the parameters of the second ejaculates, that the first ejaculates contain a high percentage of the sperms that has been mature for a long time or may have died soon, while the second ejaculates contain a lower percentage of these sperms, so its characteristics are better.

When the frequency of collection was increased to three times a week, it was observed that the sperm motility parameters, including the percentage of MS, were clearly reduced. Probably, these results were obtained due to the lower percentage of mature sperms in the third ejaculates and appearance of immature sperms in the ejaculates, which negatively affected the motility parameters (Oyeyemi and Akusu, 1998). This result confirms the results of Oyeyemi et al. (2000) which was suggested that the semen collection in bucks must not exceed two ejaculates per week.

Sperm motility parameters of the second ejaculates after dilution with a dilute containing 10% of egg yolk were significantly higher than those of the first ejaculates especially when the ejaculates

have collected twice a day, this result can be attributed that the second ejaculates have a small amount of the bulbourethral gland secretion in comparison to the first ejaculates which were consumed the most amount of bulbourethral gland secretion.

CONCLUSION

There are effects of semen collection frequency on the ability of buck semen dilution, and the best results were when ejaculates had collected twice a day at an interval of one hour, accordingly we recommended this protocol for preparation of buck semen for artificial insemination.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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