

# Pilot study for evaluating a practical method of diluting goat epididymal semen with seminal plasma

Luke Duckworth, Stan Bychawski  
Southern Veterinary Services, Tulsa, OK, USA

## Abstract

The untimely death of a sire often leads to the need for a postmortem semen collection for advanced reproductive techniques. Even with careful attention to detail, postmortem semen inherently has low motility.<sup>1</sup> Postmortem semen is collected from the epididymis and is void of seminal plasma, which is responsible for nurturing sperm and aiding in motility and maturation. However, sperm are not mixed with seminal plasma from the accessory sex glands until ejaculation. This study describes a practical method of adding seminal plasma to epididymal semen to improve semen motility. Kiko goats were anesthetized and fresh semen was collected via electroejaculation. Goats were then surgically castrated and semen was collected from the epididymis to mimic a postmortem semen collection. Seminal plasma supernatant from the fresh collections was used to dilute the epididymal semen; it increased sperm velocity and amplitude of lateral head movement.

**Keywords:** Postmortem, goat epididymal semen, seminal plasma, motility

## Introduction

The need to preserve semen from a sire at the time of death is challenging because postmortem semen is usually of low quality due to poor motility.<sup>2</sup> Postmortem semen is collected from the epididymis tail where sperm are stored before developing progressive motility.<sup>3</sup> These epididymal sperm are motile and have a flagellum, but swim in a unidirectional pattern.<sup>4</sup> Progressive motility is not initiated until sperm are mixed with seminal plasma<sup>5</sup> (SP) from the accessory sex glands during ejaculation. Due to its composition of proteins and metabolites, SP is essential for sperm maturation, nutrition, and motility.<sup>6</sup> Without SP, epididymal semen has lower fertilization rate than ejaculated semen due to inferior motility.<sup>7</sup>

A practical method to improve the motility of postmortem sperm would be valuable to any animal industry that utilizes advanced reproductive techniques. Sperm function, fertilization, and embryo development are all influenced by SP.<sup>8</sup> Thus, we hypothesized that addition of SP to an epididymal semen sample increases sperm motility (as demonstrated in human semen).<sup>4</sup> Commercial synthetic SP is not available and thus SP needs to be collected and processed. In postmortem collection, SP from a live sire can be used as a diluent for epididymal semen. Alternatively, SP from a previous live collection

can be filtered and frozen for use as needed. This can be performed in a field or clinical setting.

## Materials and methods

Initial experimental design included comparing epididymal semen and seminal plasma diluted epididymal (SPDE) semen to fresh semen. Given the cost of intact male breeding stock, a pilot study was conducted to evaluate the experimental design for a practical method of adding SP to a postmortem semen sample to increase sperm motility. This project was also intended to teach 4<sup>th</sup> year veterinary students at Oklahoma State University how to perform a fresh semen collection, surgical castration, and postmortem semen collection. In accordance with OSU IACUC policy, this project was exempt because it utilized tissues collected from animals for a routine breeding soundness examination that occurred for teaching purposes.

Five Kiko goat kids (8 months of age and ~45 kg) were brought to Oklahoma State University teaching hospital for semen collection followed by castration in October (Northern Hemisphere). The first kid was used as a demonstration. Each of 4 veterinary students then performed semen collection, surgical castration, and epididymal semen collection on the

remaining kids. Food and water were withheld for 12 hours in preparation for surgery. Kids were manually restrained and the neck was clipped and prepped with a surgical blade and 70% isopropyl alcohol. The jugular furrow was occluded at the left thoracic inlet and 0.05 mg/kg intravenous xylazine (Xylamed, VetOne, Boise, Idaho) and 0.25 mg/kg ketamine (Ketamine 200, Axtell, Pilot Point, Texas) were given in the left jugular vein.

Once anesthetized, kids were placed in left lateral recumbency. Digital pressure was applied to sigmoid flexure and prepuce to exteriorize the penis. Penis was grasped, cleaned, and isolated with gauze sponges. Rectum was evacuated of fecal pellets before introducing the 2-electrode rectal probe. Electrical stimulation was provided via a Pulsator V electroejaculator. The electroejaculator was operated in manual mode. A series of electrical impulses in increasing intensity was applied from levels 1-3. Semen was collected into a glass tulip flask. Fresh semen was diluted 1:1 with Triladyl (Minitube, Tiefenbach, Germany) and a 25  $\mu$ l portion of the sample was transferred to a Falcon tube as a control (Fresh). The remaining sample was transferred into a 15 ml conical vial and centrifuged for 10 minutes at 895 g. The SP supernatant was filtered through a 0.22  $\mu$ m syringe filter and saved to mix with the epididymal sample. Fresh semen served as an internal control for each subject.

After semen collection, kids were placed dorso-ventrally on a surgery gurney. Scrotal neck was isolated manually and pressure was applied to tighten the scrotum around testis. Scrotum was clipped and aseptically prepped with 4% chlorhexidine and 70% isopropyl alcohol and 60 mg of 2% lidocaine was used to block each spermatic cord. The distal half of the scrotum was removed via excision with a number 22 scalpel blade. Parietal vaginal tunic was excised over epididymal tail. Care was taken not to excise the epididymis. Testes were then exteriorized through the parietal vaginal tunic, leaving the visceral vaginal tunic intact. The fascia surrounding the spermatic cords was stripped and pushed dorsally. A Reimer emasculator was applied around the spermatic cord between the head of the epididymis and the scrotal incision. Spermatic cord was crimped to aid in hemostasis. After 2 minutes of crimping, the spermatic cord was severed on the distal side of the emasculator. A pair of curved hemostats was secured to the spermatic cord before the emasculators were released and observed for proper hemostasis. Spermatic cord was released when no hemorrhage was observed. Similar procedure was repeated for the other testis.

Castrated testes were held firmly in hand, so the tail of the epididymis was accessible. Number 22 scalpel blade was used to gently mince epididymis tail repeatedly until the tissue began to disintegrate. Care was taken not to contaminate the epididymal semen sample during mincing. Scalpel blade was used to scrape and collect the semen sample as the epididymis was sliced. Semen was then rinsed off the scalpel blade into a 15-ml conical vial, using Triladyl egg yolk extender. These steps were repeated until the epididymal tail was completely sliced. Extended semen sample was then centrifuged for 10 minutes at 895 g. The supernatant was removed and 25  $\mu$ l semen samples were placed in 2 Falcon tubes. Then, 25  $\mu$ l of Triladyl extender was added to 1 epididymal sample to mimic a postmortem sample (Epididymis), whereas 25 ml of SP was added to the other epididymal sample creating a SPDE sample (50% Seminal). Each sample was evaluated using computer assisted sperm analysis (CASA). Data were analyzed using a paired Student's t-test.

## Results

### Velocity

Velocity average pathway (VAP) measures the average velocity of sperm travel in m/s. Compared to epididymal samples, mean progressive VAP increased in 3 of 50% seminal samples, remained the same in 1, and slightly decreased in 1 sample. Velocity straight line (VSL) measures the straight-line distance a sperm travels between 2 scans in m/s. Mean progressive VSL increased in 3 of 50% seminal samples, remained the same in 1, and slightly decreased in 1 sample. Velocity curvilinear (VCL) measures sperm velocity over the path traveled and indicates cell vigor in m/s. Mean progressive VCL increased in 4 of 50% seminal samples and slightly decreased in 1 sample. Progressive VAP, VSL, and VCL all were higher ( $p > 0.5$ ) for the 50% seminal samples compared to epididymal samples.

### Motility

Progressive sperm number was higher ( $p > 0.05$ ) in 4 of the 50% seminal samples compared to epididymal samples (Table 1).

### Head movement

Amplitude of lateral head displacement (ALH) measures the height/width of head movement and beat cross frequency (BCF) measures the frequency of those movements in  $\mu$ m and Hz, respectively (Table 2). Compared to epididymal samples, ALH increased in 3 of 50% seminal samples, stayed the same in 1 sample, and slightly decreased in another sample. BCF stayed relatively unchanged in 3 of 50% samples, slightly increased in 1 sample and slightly decreased in another sample. All 50% seminal samples had a lower ( $p > 0.5$ ) BCF than fresh collections.

## Discussion

Seminal plasma increased the velocity and amplitude of lateral head movement of epididymal semen when used as a diluent. The original design for this project was to use each goat as its own control. The fresh collection served as an internal control and allowed veterinary students to perform a semen collection on an anesthetized ruminant. The epididymal and SPDE samples could be compared against the fresh collection.

Two problems lead the authors to conclude a fresh collection is not necessary in a larger study. First, not every kid gave a usable fresh collection. Second, semen parameters for the ejaculates were poor. Some fresh semen collections had lower motility metrics than their corresponding SPDE samples. We theorize kids were entering puberty and that resulted in lower semen quality than would be expected for mature bucks. Given the goal to teach castration and semen collection to veterinary students, young cull bucks were the only animals economically affordable. Mature breeding bucks were too high value for the project and producers were uncomfortable selling breeding bucks for a project that included castration. Due to the problems encountered, a fresh collection will not be part of the methods in further studies. Also, buying enough intact mature animals to castrate is not economical. In future studies, testes will be utilized from a harvest facility. Furthermore, only an epididymal sample and SPDE sample will be evaluated. This will also allow a larger sample size.

**Table 1.** Progressive motility metrics before and after adding caprine seminal plasma to caprine epididymal semen

Animal ID	Sample type	Progressive count (x 10 <sup>6</sup> )	Mean progressive VAP <sup>a</sup> (m/s)	Mean progressive VSL <sup>b</sup> (m/s)	Mean progressive VCL <sup>c</sup> (m/s)	p value
1	Fresh	101	119.01	106.96	193.92	—
1	Epididymis	321	205.40	191.73	244.46	0.077
1	50% Seminal	412	220.86	205.17	266.23	
2	Fresh	N/A	N/A	N/A	N/A	—
2	Epididymis	266	232.81	215.84	296.11	0.167
2	50% Seminal	293	231.33	215.35	301.64	
3	Fresh	379	120.91	112.83	174.28	—
3	Epididymis	649	211.95	196.67	258.29	0.214
3	50% Seminal	322	216.36	199.51	272.55	
4	Fresh	N/A	N/A	N/A	N/A	—
4	Epididymis	239	189.63	176.04	243.15	0.173
4	50% Seminal	642	199.99	187.62	254.56	
5	Fresh	888	146.85	140.36	175.96	—
5	Epididymis	584	188.65	177.73	221.12	0.381
5	50% Seminal	599	179.94	169.25	215.79	

<sup>a</sup>Velocity average pathway  
<sup>b</sup>Velocity straight line  
<sup>c</sup>Velocity curvilinear

**Table 2.** Head movement metrics before and after adding seminal plasma to epididymal semen

Animal ID	Sample type	Progressive mean ALH <sup>e</sup> (µm)	Progressive mean BCF <sup>f</sup> (Hz)	p value
1	Initial	6.64	41.13	—
1	Epididymis	8.02	25.83	0.382
1	50% Seminal	9.68	25.10	
2	Initial	N/A	N/A	—
2	Epididymis	11.21	23.98	0.442
2	50% Seminal	12.18	23.31	
3	Initial	7.78	32.65	—
3	Epididymis	9.88	25.82	0.271
3	50% Seminal	9.76	27.57	
4	Initial	N/A	N/A	—
4	Epididymis	9.10	27.81	0.472
4	50 Seminal	10.98	25.55	
5	Initial	7.01	28.36	—
5	Epididymis	9.10	24.77	0.423
5	50% Seminal	8.64	25.53	

<sup>e</sup>amplitude of lateral head displacement  
<sup>f</sup>beat cross frequency

Each fresh semen sample collected was centrifuged and the SP supernatant was used to dilute that buck's epididymal sample. This was problematic for 2 kids that did not give a fresh semen sample. Their epididymal samples were mixed with SP from Animal 3. The goal of this project was to provide a real-world option of improving postmortem semen quality. Seminal plasma will not be available at postmortem collection; therefore, prepared SP from another sire can be used. In a larger study, we intend to perform fresh semen

collections in the same species, mix them together, and use a 'homogenous donor' SP preparation to dilute the epididymal semen in the experimental group. This would provide a uniform dilution agent, since SP quality differs among SP donors.<sup>9</sup>

Motility of epididymal semen is pH dependent.<sup>2</sup> Raw epididymal semen is too dense and has to be diluted for CASA evaluation; Triladyl was used to rinse epididymal semen off the

scalpel and thus further diluted the epididymal semen. Bovine epididymal semen has a pH of 5.8.<sup>2</sup> It is assumed that caprine epididymal semen has a similar pH. Triladyl has a neutral pH of 6.9 and increases the pH of the epididymal sample. Motility increases very rapidly when pH increases.<sup>10</sup> Therefore, the baseline analysis of the Triladyl diluted epididymal semen was likely artificially elevated. Sterile saline has an acidic pH similar to epididymal semen and will be used as the dilution agent for the control sample in future studies.

This pilot study supported the hypothesis that adding SP to epididymal semen increases sperm velocity. Previous studies had an increase in VAP and VSL and a decrease in VCL for ejaculated sperm compared to epididymal sperm.<sup>3</sup> Increase in velocity on an average pathway and in a straight-line favor increases progressive motility. An increase in velocity along a curved line indicated that SP diluted epididymal semen still followed a curved line, but at a higher velocity. We concluded that increased velocity in all measured categories was evidence that adding SP to epididymal semen increased motility.

Addition of SP to epididymal semen increased ALH, and decreased BCF; ALH and BCF are important for fertilization and sperm/ovum interaction.<sup>11</sup> A higher ALH indicates more sperm cell vigor and favors progressive motility, whereas a lower BCF results in less frequent sperm head movement. A lower BCF increases proximity between the sperm and ovum that ultimately increases the chance for fertilization.<sup>11</sup> Sperm movement can be classified in 2 phases: progressive mode and attack mode.<sup>11</sup> Progressive mode occurs in the larger tubular areas of the female reproductive tract and is associated with higher velocity and ALH of the sperm. Attack mode occurs in the smaller tubular area of the female tract (oviduct) and is characterized by a lower BCF to facilitate proximity between the sperm and ovum. Therefore, an increase in ALH would likely favor progressive motility whereas a decrease in BCF would favor sperm/egg contact time.

The trends observed in changes in sperm velocity, ALH, and BCF indicate that expanding to a larger sample size is justified. Although the p values were not significant, literature supports the increase of sperm motility when SP is added to epididymal semen. A larger sample size should produce a significant outcome in future studies. Increasing the quality of postmortem semen samples at death is only relevant for current and future sires. However, the problem with low-quality postmortem semen still remains for sires that have already expired and had their semen frozen after death. The dilution of postmortem semen with SP is a practical approach that could also improve the quality of frozen-thawed semen. Authors will continue this study on a larger scale and also apply frozen-filtered SP to postthaw postmortem semen samples to evaluate the impacts on sperm kinetics.

## Acknowledgment

Authors thank Dale Kelley of Oklahoma State University School of Veterinary Medicine for assistance with CASA.

## Funding

Provided by Luke Duckworth and Southern Veterinary Services, Tulsa, OK.

## References

1. Monteiro GA, Papa FO, Zahn FS, et al: Cryopreservation and fertility of ejaculated and epididymal stallion sperm. *Anim Reprod Sci* 2011;127:197-201. doi: 10.1016/j.anireprosci.2011.08.002
2. Acott TS, Carr DW: Inhibition of bovine spermatozoa by caudal epididymal fluid: II. Interaction of pH and a quiescence factor. *Biol Reprod* 1984;30:926-935. doi: 10.1095/biolreprod30.4.926
3. Goovaerts IGF, Hoflack GG, Van Soom A, et al: Evaluation of epididymal semen quality using the Hamilton–Thorne analyser indicates variation between the two caudae epididymides of the same bull. *Theriogenology* 2006;66:323-330. doi: 10.1016/j.theriogenology.2005.11.018
4. Lindholmer CH: The importance of seminal plasma for human sperm motility. *Biol Reprod* 1974;10:533-542. doi: 10.1095/biolreprod10.5.533
5. Carr DW, Acott TS: Inhibition of bovine spermatozoa by caudal epididymal fluid: I. Studies of a sperm motility quiescence factor. *Biol Reprod* 1984;30:913-925. doi: 10.1095/biolreprod30.4.913
6. Wang F, Yang W, Ouyang S, et al: The vehicle determines the destination: the significance of seminal plasma factors for male fertility. *Int J Mol Sci* 2020;21:8499. doi: 10.3390/ijms21228499
7. Chaveiro A, Cerqueira C, Silva J, et al: Evaluation of frozen thawed caudal epididymal sperms and in vitro fertilizing potential of bovine sperm collected from the caudal epididymal. *Iran J Vet Res* 2015;16:188. doi: 10.22099/ijvr.2015.3054
8. Juyena NS, Stellate C: Seminal plasma: an essential attribute to spermatozoa. *J Androl* 2012;33:536-551. doi: 10.2164/jandrol.110.012583
9. Turunen T, Magris M, Malinen M, et al: Seminal-plasma-mediated effects on sperm performance in humans. *Cells* 2022;11:2147. doi: 10.3390/cells11142147
10. Carr DW, Acott TS: Intracellular pH regulates bovine sperm motility and protein phosphorylation. *Biol Reprod* 1989;41:907-920. doi: 10.1095/biolreprod41.5.907
11. Raveshi MR, Abdul Halim MS, Agnihotri SN, et al: Curvature in the reproductive tract alters sperm–surface interactions. *Nat Commun* 2021;12:3446. doi: 10.1038/s41467-021-23773-x