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Effect of warmed semen extender on boar sperm quality post-collection

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Abstract: *Semen used for artificial insemination (AI) in the swine industry is typically collected into a warmed semen collection cup containing an empty collection bag. If the ambient temperature does not closely match the temperature of the warmed collection cup and semen at the time of collection then negative effects to the motility and morphology of the sperm cells may occur due to temperature shock. The purpose of this research was to determine if collecting boar semen directly into semen extender warmed to 38.5°C would affect sperm quality post-collection. Sexually mature Berkshire x Duroc crossbred boars (n = 7) were semen collected once per week for four consecutive weeks. Every other collection, the boar's ejaculate was collected into a collection cup and plastic collection bag warmed to 38.5°C containing either no semen extender (control) or 100 mLs of a commercially available long-term semen extender warmed to 38.5°C (treatment). Following collection and processing, the semen was extended to 37.5 × 10^6 sperm/mL and stored for 6 days post-collection in a semen cooler at 17°C. Motility and morphology were evaluated on day 0 (day of collection) and day 6. There was no day x treatment effect (P > 0.05). Statistical differences (P = 0.03) were found between the treatment and control for sperm motility (82.2 vs. 75.2%) and sperm progressive motility (64.1 vs. 53.5%). No differences (P = 0.96) were present for normal sperm morphology in the treatment compared to the control (89.1 vs. 89.0%). These data suggest that boar semen ejaculates collected into a collection cup and plastic collection bag containing 100 mLs of semen extender warmed to 38.5°C will have greater percentages of motile and progressively motile sperm compared to boar sperm collected into a collection cup and plastic collection bag warmed to 38.5°C containing no semen extender.*

Key words: *boar*, *sperm*, *semen extender*, *sperm motility*, *sperm morphology*

1. Introduction

With current boar semen collection protocols, the semen is normally collected into a collection cup that has been heated to a temperature that closely matches the boar's body temperature (approximately 38°C; Flowers, 2017) in order to prevent temperature shock to the sperm cells. Temperature shock is a possibility at the time of semen collection because the semen coming from the boar will be at body temperature, but the pre-heated collection cup may not be able to maintain its heated temperature because the boar generally takes 5 to 10 minutes to complete ejaculation. As a result, the loss of temperature of the pre-heated collection cup may lead to cold temperature shock of the sperm cells, depending on the ambient temperature. This cold temperature shock may be detrimental to the integrity of the sperm cell plasma membrane and result in a hastened degradation of the plasma membrane during the storage period post-collection (Paulenz et al., 2000; Purdy et al., 2010; Jung et al., 2015) and result in compromised semen quality (White, 1993). Recent research by Rodriguez et al. (2012) and Schulze et al. (2013) has demonstrated that the temperature of the semen on the day of collection and processing can potentially have an adverse effect on the sperm quality during the storage time period. A possible solution to the loss of temperature of the pre-heated collection cup is to have the cup contain semen extender in the semen collection bag that has been warmed to same body temperature of the boar. Due to the higher specific heat capacity of a liquid compared to air, collecting the semen directly into the warmed semen extender may alleviate the temperature shock that can occur to the sperm cells. Therefore, research into the effect of collecting semen into warmed semen extender on sperm quality post-collection is warranted.

2. Materials and Methods

Animal breeding, housing, feeding and semen collection training

All procedures with animals were approved by the University of Central Missouri Institutional Animal Care and Use Committee (IACUC# 533).

Seven crossbred (Berkshire x Duroc) male piglets were used. Male piglets were weaned at 22 days of age. During the 19 week nursery/grower/finishing phase, male piglets were housed in 1.8m by 2.8m pens and provided *ad libitum* access to feed and water. At approximately 160 days of age, the young adult boars were moved to individual pens (5m x 3m) in a curtain-sided, concrete-floor, naturally-ventilated, high-tunnel building. Supplemental ventilation for cooling during the summer months was provided by fans and a mist-cooling system that was activated when ambient temperatures reached 26°C. The young adult boars were provided *ad libitum* access to water and were hand-fed daily 2.5 kg each of a 13% crude protein diet formulated to meet all National Research Council (NRC) requirements for growing boars (NRC, 2012). Between 160 and 190 days of age, the boars were trained to collect from a dummy sow. At 202 days of age the trained boars were placed on a once per week collection frequency.

Experimental design

Semen collection treatment: The boars were collected on the same day, once per week, during each four-week replication of the study. The first replication was performed during four weeks in August of 2016 (n = 4) and the second replication during four weeks in September of 2017 (n = 3) in Warrensburg. Missouri, USA. Boars were collected in the morning between 0800 and 1000 during which the average ambient temperature was 30.5°C. Each boar was collected four times (i.e. once per week for four consecutive weeks). Every other collection, the boar's ejaculate was collected into a collection cup (Thermos™, Schaumburg, IL) and plastic collection bag warmed to 38.5°C that contained either an empty collection bag containing no semen extender (control) or a collection bag containing 100 mLs of warmed semen extender. This method of semen collection allowed for each boar to act as its own control for the study. During each week of collection, approximately half of the boars were collected into a collection bag containing the warm semen extender and half of the boars were collected into an empty collection bag. The following week this collection procedure was reversed. This procedure was repeated twice for each four-week replication. The semen extender was maintained at 38.5°C until just prior to collection by storing the semen extender in a laboratory oven (Boekel Scientific, model # 107800, Feasterville, PA), located at the boar collection facility.

Preliminary research to determine the amount of warmed semen extender to include in the collection bag: Preliminary research tested whether 50, 100 or 150 mLs of semen extender should be included in the collection bag at the time of collection. Using 150 mLs of semen extender consistently resulted in a concentration of sperm that was too dilute to extend to a concentration of 37.5×10^6 sperm/mL, which is the swine industry standard concentration used in artificial insemination (AI) doses. There was a slight numerical advantage in motility and progressive motility of 100 mLs compared to 50 mLs (data not shown). Therefore, a volume of 100 mLs of warm semen extender in the semen collection bag was used in the study.

Ejaculate collection and processing

Ejaculates were collected using the gloved-hand technique and standard semen collection protocols and equipment (Almond et al., 1998). Immediately following collection the ejaculates were transported to the laboratory where sperm analyses were performed. The volume of semen was estimated by weighing the collection bag for each ejaculate and using the conversion factor of 1 g semen equivalent to 1 ml volume. Concentrations of sperm were evaluated using a self calibrating photometer (Sperma-Cue™, Minitube of America, Verona, WI). Total number of sperm was calculated by multiplying the volume by the concentration. Following determination of semen concentration, the ejaculate was extended to a final concentration of 37.5×10^6 sperm/mL using a commercially available long-term extender (EnduraGuard Plus; Minitube of America, Verona, WI). The samples were allowed to cool to room temperature (~20 to 21°C) for 15 minutes and then placed in a semen cooler at 17°C (Minitube of America, Verona, WI) for six days post-collection.

Semen analysis

The semen samples were analyzed for motility and morphology on day 0 (i.e. day of collection) and day 6 post-collection. Percentages of motile sperm, progressively motile sperm and percentages of morphologically normal and abnormal sperm were evaluated.

Sperm motility: Motility analyses were performed using Sperm Vision™ software (Minitube of America, Verona, WI) on images obtained by a digital camera attached to phase contrast microscope (Axio Scope.A1; Zeiss, Oberkochen, Germany). Prior to analysis, a 1 mL aliquot was incubated at 37°C for 30 minutes. Next, a 20 μL droplet was placed into a evaluation chamber on a pre-warmed glass analysis slide (20 μ chamber height; ISASD4C-L; PROiSER; Valencia, Spain). Seven fields were analyzed per chamber per boar. Approximately 300 to 600 total cells per sample were evaluated.

Sperm morphology: Evaluation of sperm morphology and cellular particles was performed using a phase contrast microscope (Revelation model; LW Scientific; Lawrenceville, GA) and a 40x objective. A 50 μL aliquot from each sample was fixed by adding 1250 μL of 5% buffered formalin. The analysis consisted of classifying all intact sperm within the

triple lines of a hemocytometer. A minimum of 400 sperm cells were evaluated per sample. Sperm were classified according to Almond et al. (1998) and Lovercamp et al. (2007). Intact sperm were classified into one of the following six morphological groups: normal, abnormal head, abnormal tail, proximal cytoplasmic droplet, distal cytoplasmic droplet and the distal midpiece reflex with a cytoplasmic droplet.

Statistical analysis

All statistical analyses were performed with analysis of variance procedures for mixed models (Littell et al., 1998) using the mixed procedure of SAS (SAS Ins., Cary, NC). The variance/covariance structure used for the repeated measures analyses was determined by finding the appropriate structure with the lowest fit statistics. Prior to analysis, all percentage data were normalized with an arcsine transformation (Snedecor and Cochran, 1989). Results for percentage data are reported as non-transformed means ± standard error of the mean. All other data are reported as least squares means ± standard errors. For all models, the Tukey-Kramer mean separation procedure was used to determine differences among means of independent variables when significant effects were observed.

Data for ejaculate characteristics of boars during the semen collection phase were analyzed as a complete randomized design using analysis of variance procedures for repeated measures (Gill and Hafs, 1971). Boar was considered the experimental unit. The model included week (week 1 to 4), boar (boar 1 to 7) and the interaction. The variance/covariance structure used was unstructured. The error term used to test treatment effects was boar. Week was considered the repeated measure.

Data for sperm motility and morphology were analyzed as a complete randomized design using analysis of variance procedures for repeated measures. Ejaculate was considered the experimental unit. The model included semen extender (yes, no) and day of storage (day 0, day 6) and all appropriate interactions. The variance/covariance structure used was unstructured. The error term used to test for treatment effects was boar nested within the week x day interaction. Day of storage was considered the repeated measure.

3. Results and Discussion

Ejaculate analyses for semen volume, concentration, total sperm and temperature of the semen immediately following collection

Ejaculate characteristics for semen volume (168 ± 12 mLs), concentration (305 ± 32 × 10^6 sperm / mL) and total sperm (55 ± 8 × 10^9 sperm) in the ejaculate during the semen collection phase were not different ($P > 0.05$) between week, boar or the week x boar interaction (individual week and boar data not shown). The ejaculate characteristics of the boars in this study are within normal physiological ranges for boar semen ejaculates (Almond et al., 1998). There was no difference ($P = 0.17$) in the temperature of the semen immediately following collection between the control (37.2°C) and treatment (38.0°C). The ambient temperature in the naturally ventilated swine facility averaged 30.3°C during the time of collection.

Table 1. Effect of day on sperm motility parameters.

	Day of analysis post-collection	
	Day 0	Day 6
Motility parameter, %		
Total motility	83.3 ± 1.6[a]	74.2 ± 3.5[b]
Progressive motility	64.2 ± 3.0[a]	53.3 ± 3.4[b]

The data was arcsine transformed for statistical analysis. The data reported are arithmetic means and standard errors.
[ab]Means within a row without a common superscript are different ($P < 0.05$)

Sperm quality analyses for sperm motility and morphology

Motility analysis: There was no day x treatment effect for total sperm motility ($P = 0.19$) or progressive sperm motility ($P = 0.21$). Sperm motility data by day of analysis post-collection is presented in Table 1. There was a decrease ($P < 0.05$) for total sperm motility and for progressive sperm motility from day 0 to day 6 post-collection. This decrease in total sperm motility and for progressive sperm motility over six days storage post-collection has been previously documented and is expected since sperm typically decline in motility over time post-collection during storage (Paulenz et al., 2000; Lovercamp et al., 2013).

Sperm motility data by collection treatment is presented in Table 2. Sperm that were collected into 100 mLs of semen extender warmed to 38.5°C (treatment) displayed a greater ($P < 0.05$) percentage of total sperm motility and progressive sperm motility compared to sperm collected into an empty collection bag containing no semen extender (control). Previous research evaluating the effect of temperature on sperm has indicated that maintaining isothermic semen extender temperature can result in higher sperm quality (Schulze et al., 2013). The results of the present study suggest that collecting semen into semen extender warmed to 38.5°C

Table 2. Effect of semen collection treatment on sperm motility and morphology. Semen was collected into a collection cup and plastic collection bag warmed to 38.5°C containing either no semen extender (control) or a collection bag with 100 mLs of semen extender warmed to 38.5°C (treatment).

	Semen collection treatment	
	Semen collected into semen extender (treatment)	Semen not collected into semen extender (control)
Motility parameter, %		
Total motility	82.2 ± 2.9[a]	75.2 ± 2.6[b]
Progressive motility	64.1 ± 3.3[a]	53.5 ± 3.2[b]
Morphological parameter[1], %		
Normal	89.1 ± 2.2	89.0 ± 2.1
Proximal cytoplasmic droplet	1.5 ± 0.4	1.2 ± 0.2
Distal cytoplasmic droplet	3.2 ± 0.3[a]	2.4 ± 0.3[b]
Distal midpiece reflex[2]	3.6 ± 1.5	5.3 ± 2.1
Total attached cytoplasmic droplets	8.4 ± 2.1	8.9 ± 2.2
Abnormal tail	1.43 ± 0.2	1.11 ± 0.1
Abnormal head	0.93 ± 0.1	1.04 ± 0.1
Total abnormal	10.7 ± 2.3	11.0 ± 2.2

The data was arcsine transformed for statistical analysis. The data reported are
arithmetic means and standard errors.
[1]Definitions: Total attached cytoplasmic droplets (proximal + distal + distal midpiece reflex); Total abnormal (total attached cytoplasmic droplets + abnormal tail + abnormal head)
[2]The distal midpiece reflex sperm contained a cytoplasmic droplet in the midpiece reflex
[ab]Means within a row without a common superscript are different ($P < 0.05$)

maintained isothermic conditions for the sperm cells from the boar's body to the collection vessel. In other words, collecting the semen directly into the warmed semen extender prevented cold temperature shock to the sperm cells. This could explain the higher percentages of total and progressive sperm motility seen for sperm collected into the warmed semen extender.

The higher percentages of sperm cells displaying total and progressive sperm motility is intriguing and holds promise for potentially improving the ability to determine the prospective fertility of a boar's ejaculate. Research using multivariate discriminant analysis has shown that ejaculates containing sperm with higher percentages of total motility and progressive motility are associated with greater farrowing rates and total pigs born (Kummer et al., 2013). Furthermore, a study conducted by Flowers et al. (2016) evaluated heterospermic inseminations in which boar ejaculates were combined to make insemination doses in such a way that each boar was tested against all of his contemporaries in the study. The results of this study indicated that boars which consistently sired the majority of piglets demonstrated higher levels of motility and average linear sperm velocity (i.e. progressive motility). Altogether, these results from previous research coupled with the results of the present study indicate that methods to increase total and progressive sperm motility should be pursued due to their potential associated benefits of fertility. One method to accomplish this may be to collect semen from boars directly into a collection cup and collection bag containing semen extender warmed to 38.5°C as discussed in this present study.

Morphology analysis: There was no day x treatment effect for the sperm morphology categories ($P > 0.05$). There were no differences ($P > 0.05$) from day 0 to day 6 post-collection for the sperm morphology (data not shown). Sperm morphology data by collection treatment is presented in Table 2. There were no differences ($P > 0.05$) found for normal sperm, abnormal tail, abnormal head, proximal cytoplasmic droplet, distal midpiece reflex, total cytoplasmic droplets or total abnormal sperm morphology. There was a higher ($P = 0.04$) percentage of sperm cells with a distal cytoplasmic droplet in the semen collected into the semen extender compared to semen not collected into semen extender. The cause of this higher percentage of sperm with a distal cytoplasmic droplet is not known. A possible explanation for this result is that collecting into the semen extender caused the cytoplasmic droplet to move from the proximal position to the distal position. However, there was not a concomitant higher percentage of sperm with a proximal cytoplasmic droplet in ejaculates not collected into semen extender. Regardless of whether the semen was collected into semen extender or not, the morphological percentages of the sperm cells from the boar ejaculates were within the acceptable range (Almond et al., 1998) and therefore would not be considered prohibitive for use in AI programs.

4. Conclusions

Overall. these data suggest that boar semen ejaculates collected into a collection cup and plastic collection bag containing 100 mLs of semen extender warmed to 38.5°C will have greater percentages of motile and progressively motile sperm compared to boar sperm collected into a collection cup and plastic collection bag warmed to 38.5°C containing no semen extender. In addition, there appears to be no effect on

sperm cell morphology regardless of treatment. Further research should be conducted to determine if ejaculates collected into a collection cup and plastic collection bag containing 100 mLs of semen extender warmed to 38.5°C will have improved fertility as measured by farrowing rates and total pigs born.

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