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TECHNICAL NOTE: Comparison of Two Containers Used for Shipment of Stallion Semen

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Abstract

In Experiment 1, cooling rates and final storage temperatures of two commercial static cooling devices were compared during air transit utilizing internal temperature loggers. The cooling devices were shipped from Tucson, AZ to Springfield, MO and consisted of a reusable device (Equitainer I®; Hamilton Research Inc., Beverly, MA) and a disposable device (Equine Express®; Exodus Breeders Supply, York, PA). Cooling rates were compared during summer and winter shipments, as was the percentage of progressively motile sperm following transit. In Experiment 2, cooling rates were determined in four static cooling devices held for 48 h at a controlled-environmental temperature of 22°C. During actual transit, the mean and minimum internal temperatures reached were lower (P<0.0001) when shipping occurred during winter. However, internal temperatures were not different within season between containers. Neither season nor container affected the percentage of progressively motile spermatozoa 24 h post packaging. When ambient temperatures were controlled at 22°C, cooling rates from 19° to 10°C were similar between the Equitainer I® utilizing centrifugation tubes (–0.033 ± 0.002°C/min) and the Equine Express® (–0.035 ± 0.005°C/min); both of which provided slower (P<0.001) cooling than did the Clipper (–0.098 ± 0.005°C/min) and the Equitainer I® utilizing a cup-style isothermalizer (–0.077 ± 0.01°C/min). These data suggest that, during both the winter and summer months in the southwestern US, disposable cooling devices can be used to ship or store stallion spermatozoa for 24 h.

(Key Words: Stallion, Shipped Semen, Cooling Devices.)

Introduction

There has been a great deal of activity in the use of transported stallion semen, as most of the major breed registries in North America approved its use in the late 1990s. Producers have access to several commercially available cooling devices for use during the transportation of stallion semen. Initial studies were conducted with a reusable, rigid container (Equitainer®; Hamilton Research, Inc., Beverly, MA) that was reported to provide a cooling rate of –0.3°C/min and a final temperature of 4° to 5°C (Douglas-Hamilton et al., 1984). Brinkso et al. (2000) compared cooling rates and lowest temperatures reached when several reusable and disposable cooling devices were subjected to different ambient temperatures and suggested that the improved version of the original rigid device outperformed some of the disposable devices. Interpretation of these results, along with claims made by manufacturers of some shipping containers, have led to concern regarding the ability of disposable containers to maintain satisfactory cooling rates and/or storage temperatures during transportation (Blanchard et al., 2003), which may, in part, be due to information published by companies responsible for air transport of semen in an effort to prevent liability caused by damage during shipment of temperature-sensitive items such as feedstuffs and other biological materials (Casada et al., 2003). This information might lead to the conclusion that the containers would normally be subjected to temperatures ranging from –70° to 145°F. These results suggest that some of the disposable static cooling devices on the market may not be suitable for shipping semen, despite successful research and field use (Katila et al., 1997; Dawson et al., 1999; Webb and Arns, 2004). The objectives of this study were to compare the internal temperatures of two popular shipping containers during shipment from the desert Southwest to the Midwest region of the US as well as the ability of these containers to maintain the post storage motility of

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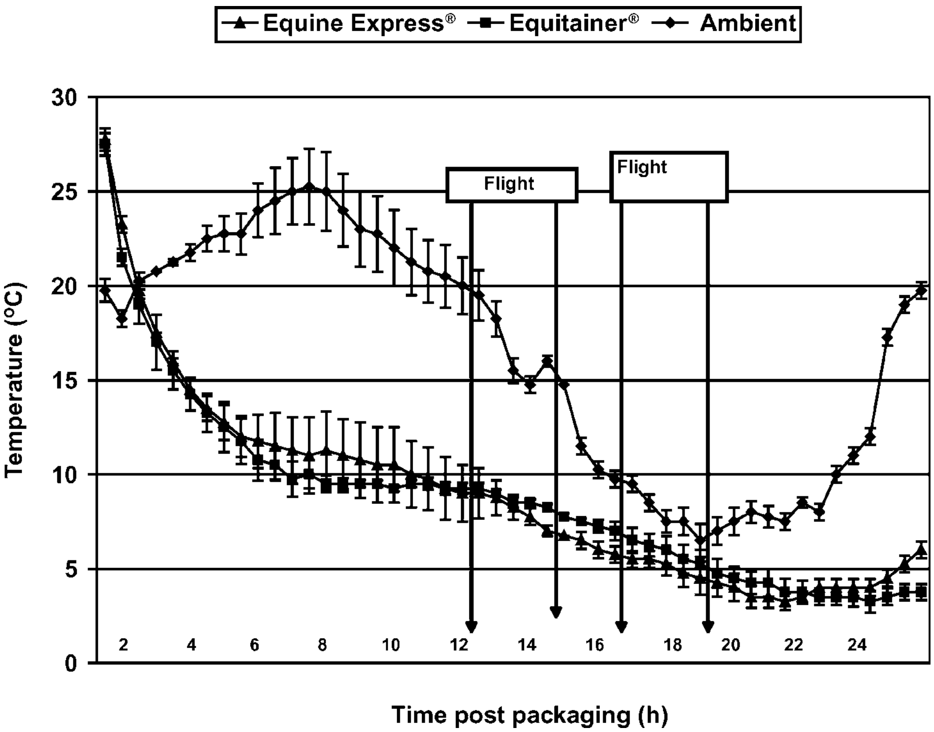

Figure 1. Ambient temperature during shipment and internal temperature of cooling devices during winter shipment. Equine Express® (Exodus Breeders Supply, York, PA) and Equitainer I®(Hamilton Research Inc., Beverly, MA).

stallion spermatozoa shipped in the containers. In the second experiment, cooling rates of two disposable and two reusable containers were compared when held under controlled ambient conditions.

Materials and Methods

Experiment 1. Semen was collected from each of two stallions and diluted at a ratio of 3:1 (extender:semen) with a commercially available stallion semen extender (Next Generation®; Exodus Breeders Supply, York, PA). Aliquots of extended semen from each stallion were packaged within each style of shipping container such that the total volume of extended semen for each container was 100 mL. Smart Button® temperature loggers (Empire Instrument, Big Bear Lake, CA) were incorporated within one of the 50-mL centrifugation tubes used to package extended semen. One of the containers was a disposable unit (Equine Express®; Exodus Breeders Supply) and the other a standard rigid/plastic device designed for multiple shipments (Equitainer I®; Hamilton Research Inc.). A temperature logger was also attached externally to the containers to measure ambient temperatures during shipment. Three sets of containers per season (February and July) were commercially shipped by overnight delivery from Tucson, AZ to Springfield, MO (FedEx Corp., Memphis, TN). Spermatozoa were evaluated for the percentage of motile spermatozoa following shipment utilizing computer-assisted sperm analysis (CEROS®; Hamilton Research Inc.).

Experiment 2. The same temperature loggers used in Experiment 1 were used to measure the cooling characteristics of four different types of containers exposed to a constant ambient temperature of 22°C for 48 h post packaging. Shipping containers used in this study included Equitainer I® with two types of isothermalizers (cup *vs* tube-type) and two disposable containers that consisted of the Equine Express® (Exodus Breeders Supply) and Clipper® (Hamilton Research Inc.). Five repetitions where run per container model. Total volume for the Equitainer I® with the cup style isothermalizer consisted of two 50-mL Whirl-pak® (NASCO, Fort

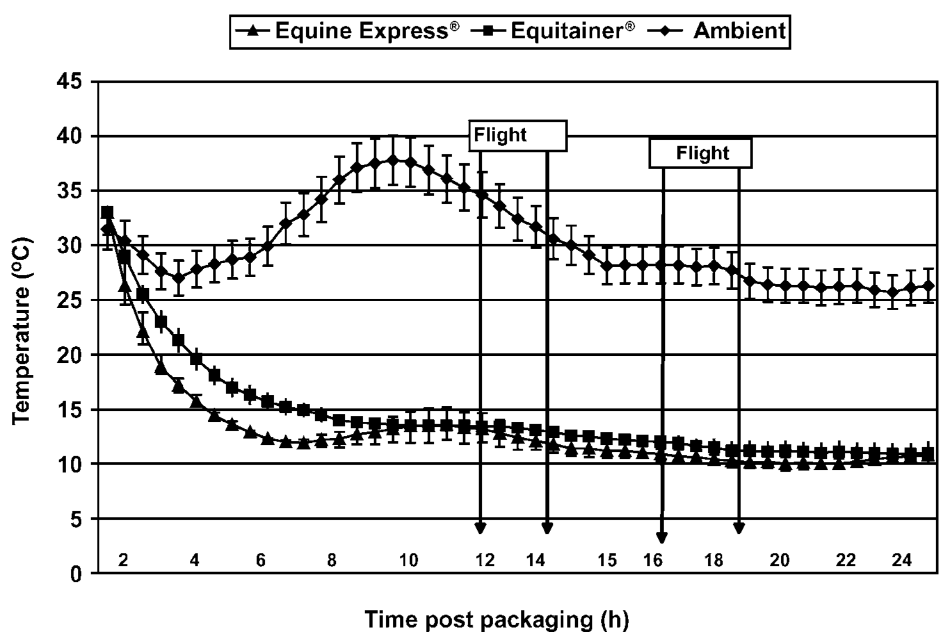

Figure 2. Ambient temperature during shipment and internal temperature of cooling devices during summer shipment. Equine Express®(Exodus Breeders Supply, York, PA) and Equitainer I®(Hamilton Research Inc., Beverly, MA).

Atkinson, WI) bags and one 60-mL ballast bag (Hamilton Research Inc.) to bring the total volume to 160 mL as per the manufacturer's recommendations. The Equitainer I® with tube style isothermalizer is designed to hold two 50-mL centrifugation tubes. Therefore, this volume was used for the remaining three types of shipping devices. A Smart Button® temperature logger was incorporated within one of the sample tubes or bags in each container.

Statistical Analysis. In Experiment 1, cooling rates for the samples that were shipped via air were analyzed by comparing both the mean internal temperature during shipment as well as the cooling rate. Statistical analysis of these data was conducted using the PROC GLM procedures of SAS® (SAS Inst., Inc., Cary, NC) A level of P=0.05 was set as the minimum level for significant difference. Mean percentages of progressively motile spermatozoa were analyzed by ANOVA. A level of P=0.05 was set as the minimum level for significant difference. In Experiment 2, when the containers were exposed to constant ambient temperatures of 22°C, cooling rates and mean lowest temperature were analyzed by ANOVA, and pair-wise comparisons where made by *t* test.

TABLE 1. Influence of ambient temperature (°C) during transport on internal temperature of commercial cooling devices. Data are presented as means ± standard deviations.

	Minimum internal temperature		Ambient temperature	
Item	**Equine Express[a]**	**Equitainer I[b]**	**Minimum**	**Maximum**
Summer shipment	10.0^x ± 0.3	10.9^x ± 0.9	25.7 ± 4.3	37.8 ± 6.7
Winter shipment	2.7^y ± 0.3	3.3^y ± 0.2	5.7 ± 1.6	25.3 ± 1.9

[a]Exodus Breeders Supply (York, PA).

[b]Hamilton Research Inc. (Beverly, MA).

[x,y]Means in the same column with different superscripts are different (P<0.001).

Results and Discussion

By comparing data from the external temperature logger with that of records from the delivery company, fluctuations in ambient temperatures can be matched with container locations during shipment. Extremes in ambient temperature occurred when containers were being transported by ground service to the airport, while the plane was awaiting takeoff, and during flight (Figures 1 and 2). The temperature ranges observed in this study were similar to those observed when the same technology was used to monitor conditions during shipment of containers from California to Kansas (Casada et al., 2003). The highest ambient temperature (45°C) to which containers were exposed during flight occurred during summer months; the lowest (4°C) occurred during winter shipment (Table 1). The extremes in potential ambient temperatures used as treatments in previous studies (Brinkso et al., 2000) were not experienced in this study during actual air shipment. In addition, variations in internal temperatures were similar between container types during both seasons (P=0.42, summer; P=0.92, winter). Moreover, neither container type nor season had an affect on progressive motility of spermatozoa following shipment (P=0.94; Table 2). This result suggests that both the disposable (Equine Express®) and reusable (Equitainer I®) cooling devices tested in this study can tolerate actual commercial shipment requiring flight. Previous research has shown that stallion spermatozoa can be cooled rapidly to 19°C and then, to reduce spermatozoal injury, must be cooled slowly (–0.1° to –0.05°C/min) from this point down to 8°C. Stallion spermatozoa can then be rapidly cooled to the final storage temperature of 4° to 5°C (Kayser et al., 1992; Moran et al., 1992). In the current study, when containers were exposed to constant temperatures of 22°C, cooling rates were within the range suggested as optimum within the aforementioned studies. In addition, final temperatures ranged from 5.6° to 8.9°C/min (Table 3), and both cooling rates and final temperatures were similar to those reported by Brinsko et al. (2000). However, when containers were evaluated during summer use in Arizona, lowest temperatures of samples stored in containers during the first 24 h never fell below 10°C in either the Equitainer I® or disposable Equine Express® (Table 3). These results agree with the previous report in that when the containers were exposed to high ambient temperatures,

TABLE 2. Mean progressive motility (%) of stallion spermatozoa after shipment. Values for motility did not differ between container type or season (P=0.94).

Item	no.	Equine Express[a]	Equitainer I[b]	Season mean
Summer	6	40.7 ± 13.0	43.0 ± 15.1	41.9 ± 13.5
Winter	6	42.3 ± 15.0	40.5 ± 14.7	41.4 ± 14.5
Container mean	12	41.5 ± 13.4	41.8 ± 13.7	

[a]Exodus Breeders Supply (York, PA).

[b]Hamilton Research Inc. (Beverly, MA).

TABLE 3. Cooling rates from 19° to 10°C and lowest temperatures reached within 24 h for different containers exposed to controlled ambient temperatures of 22°C or summer ambient temperatures in Arizona.

Item	Equitainer[a]/tube	Equitainer/cup	Equine Express[b]	Clipper
	Ambient temperature, 22°C			
Cooling rate, °C/min	–0.033^{w} ± 0.002	–0.077^{x} ± 0.010	–0.035^{w} ± 0.005	–0.098^{x} ± 0.005
Minimum temperature, °C	7.6^{y} ± 0.2	6.2^{z} ± 0.3	8.9^{y} ± 0.4	5.6^{z} ± 0.2
	Summer Arizona			
Cooling rate[c] °C/min	–0.034^{w} ± 0.001		–0.029^{w} ± 0.002	
Minimum temperature, °C	13.3 ± 0.4		10.3 ± 0.2	

[a]Exodus Breeders Supply (York, PA).
[b]Hamilton Research Inc. (Beverly, MA).
[c]Calculated from 19oC to the lowest temperature recorded if >10°C.
^{w,x}Means in the same row with different superscripts are different (*P*=0.007).
^{y,z}Means in the same row with different superscripts are different (*P*=0.021).

internal temperatures did not reach 5°C, which is the temperature that has been proposed as optimal for storage of stallion semen (Pickett, 1993; Blanchard et al., 2003). However, when the cooling devices where exposed to either controlled ambient temperatures of 22°C or actual ambient temperatures in Arizona, cooling rates through the critical range of 19° to 9°C were within the recommended range or lower (Table 3). In addition, in the aforementioned study, motility of spermatozoa was not influenced when containers were exposed to ambient temperatures of 37° or 22°C, which is in agreement with previous reports (Katila et al., 1997). However, when the containers were placed in a –70°C freezer for 6 h immediately post processing, motility of samples stored in the Equitainer I® was superior to those stored in disposable [Expecta Foal® (Parker, CO); Lane STS® (Lane Mfg., Denver, CO); Equine Express®) containers (Brinkso et al., 2000). It is not surprising that the Equitainer I® would prove superior under these conditions, as it does contain more insulation than the disposable containers. In the current study, when semen was shipped during winter months, the coldest ambient temperature occurred during ground transportation and not flight. Moreover, in Experiment 1, the coldest temperature in the cargo compartment only reached 4°C, a much higher temperature than used in the previous study to mimic possible temperature extremes during flight (Brinkso et al., 2000). Therefore, selection of devices for air shipment of semen from equines or other species should not be based on results of laboratory test in which containers were exposed to conditions that are unlikely to occur during normal shipment by air, such as constant conditions of 100°F for 24 h or –70°F during the initial phase of storage.

Implications

Variations in temperatures of shipping containers as experienced when containers were exposed to commercial transportation from Arizona to Missouri did not affect internal temperatures of the container to the extent that motility of stallion spermatozoa shipped in the containers was affected. Because of the difference in the initial investment cost [$250 (reusable) *vs* $30 (disposable)], stallion owners often required that the reusable containers be returned no later than 2 d after they are received. This results in an additional expense of $45 to $65 to the mare owner. Even if they are only used once, use of disposable containers can result in a net savings of $15 to $30 per shipment on return shipping cost alone minus the purchase cost of the containers. Finally, producers should be aware of the effects of ambient temperatures on the cooling characteristics of both reusable and disposable containers.

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