Title: Mitigation of summertime boar infertility by evaporative and conductive cooling, **NPB#16-165**

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Revised2

Industry Summary:
The impact of heat stress on sperm production and quality has been demonstrated previously. This project aimed to quantify some temperatures in commercial studs and then evaluate mitigation strategies for boars subjected to conditions seen in 'the real world'. Hobo temperature loggers were placed in ten commercial studs in IL, MO, MC, MO and TX with varying cooling systems. We anticipated air conditioned studs would have very little variation so only included one. Interestingly, some studs with cool cells were better able to maintain conditions in the thermoneutral range during the period of data collection. As expected, cool cells were generally more effective under conditions of low ambient relative humidity (Figure 5). We selected a cycle within the range observed under commercial conditions and programmed that for our environmental chambers.

Twelve boars were moved to our chambers and surgically implanted with temperature sensors in the abdominal cavity and in the scrotum. We monitored effectiveness of cooling strategies to reduce efforts to cope with heat stress (e.g. less impact on respiration rate or skin temperature) and to maintain homeostasis in the body and scrotum. Each boar rotated among all cooling strategies. Strategies tested included all combinations of fans and drippers on the neck and scrotum as well as a commercially available water cooled floor panel. Each cooling strategy worked to varying degrees. Ultimately the most effective strategy was used to provide a 9wk treatment of boars under summer conditions. The strategy most effective at internal temperature regulation was the one that had both fans and drippers on both the neck and the scrotum. The twelve boars were housed every other stall with cooling or not. At the end of 9wk boars were sacrificed and samples collected to evaluate effectiveness of this strategy to mitigate the impact of heat on sperm quality measures. Results demonstrated that cooling was effective at reducing the negative impact of elevated ambient temperatures (Table 1).

Key Findings:

- Elevated ambient temperatures observed in commercial studs lead to elevated respiration rate and skin and rectal temperature when replicated under controlled conditions.
- Interventions (drippers, fans, cooled floors) resulted in modest improvements in measured variables.
- Even with modest impact on measured variables, improvements were observed for sperm quality traits post-mortem.

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Introduction:

Most pigs have been moved indoors in part to allow mitigation of temperature fluctuations in the environment. Even so, current systems fail to completely mitigate the effects of high summer temperatures, and sperm quality and quantity have been shown to be inferior during the summer. It is known that boar studs in particular apply more rigorous environmental controls in an attempt to maintain thermoneutral conditions, with many even using air conditioning for the animal space. It is not known how effective these measures are, so applying results from scientific literature can be challenging. We were interested in what thermal conditions are boars actually exposed to during the summer in the US, and how effective are available mitigation strategies other than air conditioning at reducing the impact of these conditions?

Objectives:

1) Measure ambient temperature and humidity within selected boar studs during summer 2016.
2) Recreate average and extreme temperature and humidity conditions observed in commercial studs by using environmental chambers at the University of Missouri and then:
   a. test the effectiveness of neck and testicular drippers with and without forced air applied directly to the boar (evaporative cooling methods) by assessing both abdominal and testicular temperature of treated boars.
   b. test the effectiveness of a conductive cooling method that employs a cooled floor for boars to lie on by assessing both abdominal and testicular temperature of treated boars.
3) Assess the capacity of the most effective cooling method that we identify to maintain semen quality in boars subjected to two months of heat stress under controlled environmental conditions.
4) Determine factors related to temperature differences in commercial studs.

Materials & Methods:

Objective 1: Measure ambient temperature and humidity within selected boar studs during summer 2016

In a previous study, we placed data loggers into farrowing units to assess the ambient temperature in 4 different barns (Figure 1). We will collect similar data in boar studs during the summer of 2016. Data loggers will be placed in at least four commercial studs in at least three states. These will record temperature and humidity through July and August.

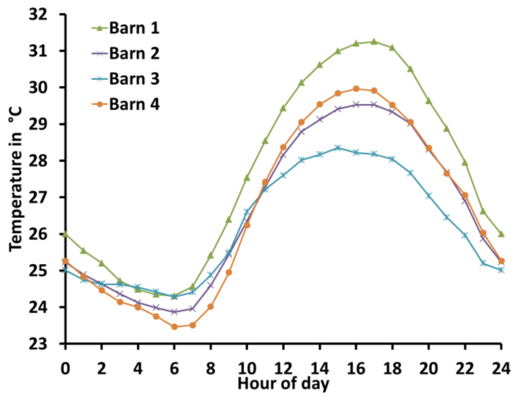
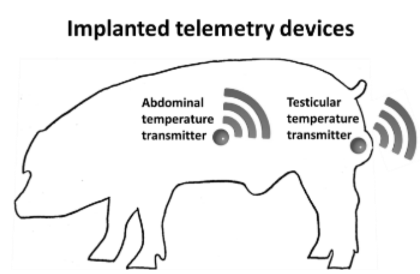


Figure 1. Average temperature inside four different farrowing units during a mid-Missouri summer. We used this information to determine chamber temperatures for subsequent studies. We will collect similar data for the proposed studies during the summer of 2016 in boar studs so that the temperatures used in the environmental chambers approximate what is seen on-farm.

Objective 2a: Recreate average and extreme temperature and humidity conditions within boar studs using environmental chambers at the University of Missouri and test the effectiveness of neck and testicular drippers with and without forced air applied directly to the boar (evaporative cooling methods) by assessing both abdominal and testicular temperature of treated boars.

Preparation of study boars: Boars will be implanted surgically with two temperature sensing telemetry devices. The first device will be placed inside a mesh bag and sutured to the abdominal omentum of the pig. The second device will be place underneath the scrotum. We plan to use Anipill Temperature Implant Model 0.1C (Data Sciences International; St. Paul, MN). The device measures both temperature and activity, weighs 1.7 g with an implant volume of 1.12 cc. It allows for continuous monitoring of temperature inside the animal. We will use surgical facilities located at the MU Animal Science Research Center to perform the surgery.

Figure 2. Location of implanted devices that continuously measure and monitor body temperature of the boars on the study.



Evaporative cooling tests. Boars will be moved into environmental chambers and held at thermoneutral (TN) for approximately one week. This TN preliminary period will be for baseline data collection. Then, the temperature/humidity cycle that simulates on-farm heat stress will be initiated. Boars will remain for approximately one week at the HS temperature/humidity without any mitigation. This HS preliminary period will be used to assess each boar's response to HS without any mitigation. We will then apply treatments in a Latin square design with seven periods. The periods will last 3 days without an intervening period. The beginning of each period will be at 6 AM (lowest temperature of the day).

Each boar will be subjected to each treatment. The airflow from the fan will be sufficient to provide a 100 ft/min air speed on the targeted areas of the boars. The drip rate from the dripper will be at least 1 gallon per hour. Treatment periods will last three days, at which time the boar will switch to a different treatment. Both abdominal and testicular temperature will be measured every 15min using a radiotelemetry device. Respiration rate and skin temperatures will be measured twice daily using a stopwatch and observing each boar for one minute (morning and afternoon).

The first Latin square will be done at a typical summer day temperature cycle. We will then increase to a more stressful temperature humidity cycle approximating a summer heat wave. The Latin square will be completed again and measurements taken. At the end of the heat wave, the room temperature will return to thermoneutral and data will be collected for an additional week. The total time on trial will be 9 weeks (1 week TN, 1 week HS without mitigation, 3 week Latin square 1 (typical summer day), 3 weeks Latin square (heat wave), 1 week TN).

Figure 3. Each boar was subjected to each combination of fans and drippers on the neck or scrotum according to the schedule below.

LATIN SQUARE SETUP

Period	1	2	3	4	5	6	7	8	9	10
Boar #1	A	B	C	D	E	F	G	H	I	J
Boar #2	B	C	D	E	F	G	H	I	J	A
Boar #3	C	D	E	F	G	H	I	J	A	B
Boar #4	D	E	F	G	H	I	J	A	B	C
Boar #5	E	F	G	H	I	J	A	B	C	D
Boar #6	F	G	H	I	J	A	B	C	D	E
Boar #7	G	H	I	J	A	B	C	D	E	F
Boar #8	H	I	J	A	B	C	D	E	F	G
Boar #9	I	J	A	B	C	D	E	F	G	H
Boar #10	J	A	B	C	D	E	F	G	H	I

Trt	Drip	Fan
A	Off	Off
B	Neck on	Neck off
C	Neck off	Neck on
D	Neck on	Neck on
E	Testis on	Testis off
F	Testis off	Testis on
G	Testis on	Testis on
H	Both	Off
I	Off	Both
J	Both	Both

Objective 2b: Recreate average and extreme temperature and humidity conditions observed within boar studs using environmental chambers at the University of Missouri and test effectiveness of a conductive cooling method that employs a cooled floor for boars to lie on by assessing both abdominal and testicular temperatures of treated boars

Conductive cooling tests. Boars will be moved into the environmental chambers and held at thermoneutral (TN) for approximately one week. This TN preliminary period will be for baseline data collection. Then, the temperature/humidity cycle simulating on-farm heat stress will be initiated. Boars will remain for approximately one week at the HS temperature/humidity without any mitigation. This HS preliminary period will be used to assess each boar's response to HS without any mitigation. We will then apply treatments in a switchback design with two periods (Figure 4). The periods will last one week without an intervening period. The beginning of each period will be at 6 AM (lowest temperature of the day).

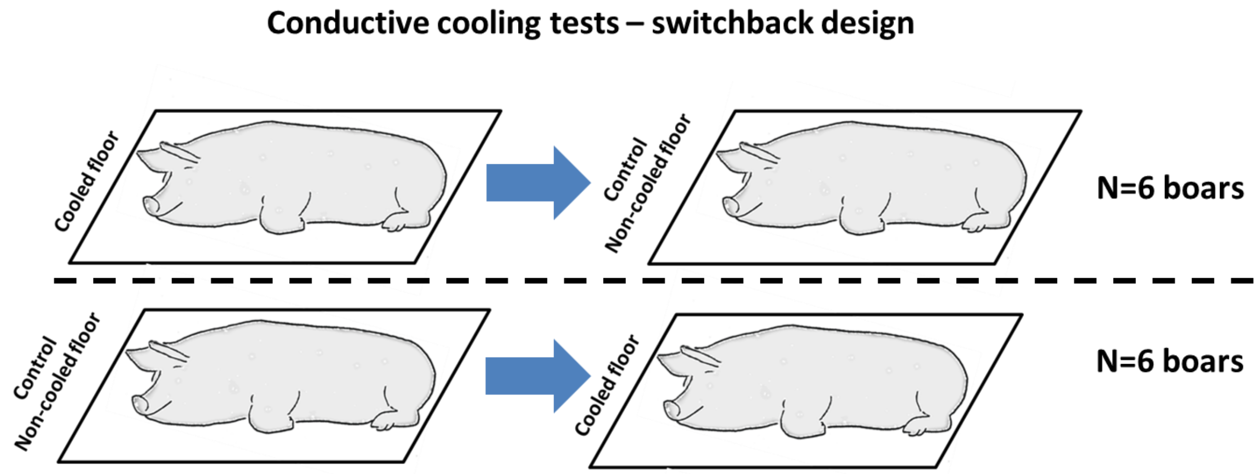


Figure 4. Switchback design for conductive cooling tests.

Conductive cooling on the floor will be provided for boars to cool them while they are resting. A minimum of 12.5 watts per square foot of cooling will need to be provided using a chilled water floor cooling system. If the technique is effective other approaches to achieve the cooling effect will be investigated further. Cooling pads will be placed on top of chamber pen floors and located such that the boars will be cooled by their neck and shoulder area.

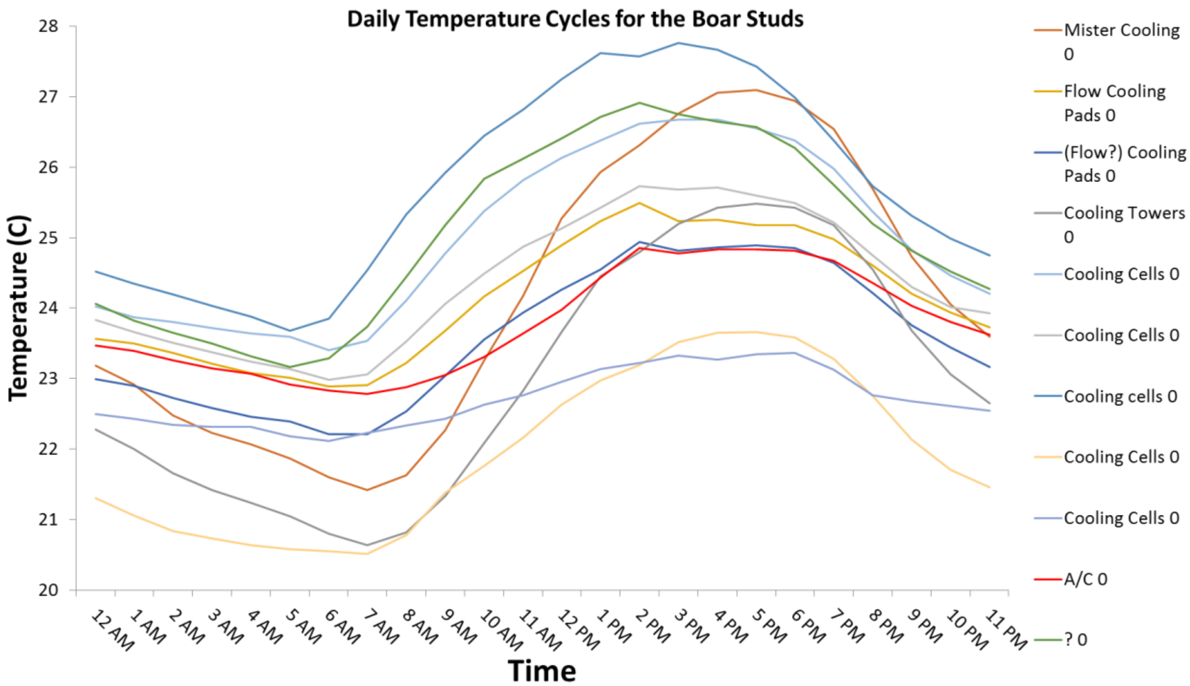
Objective 3. Assess the capacity of the most effective cooling method that we identify to maintain semen quality in boars subjected to two months of heat stress under controlled environmental conditions. Each of the mitigation approaches will be evaluated for effectiveness at reducing temperatures and respiration rate. The strategy deemed to be most effective will be tested over a complete sperm cycle. Twelve boars will be housed in stalls in an Environmental Chamber set to mimic summer conditions. Six boars will receive mitigation for 9 weeks and 6 will not. At the end of the 9 week period (one full sperm cycle plus time to replenish epidiymal stores) all boars will be slaughtered under USDA inspection and testis and epididymis collected for evaluation.

Results: Report your research results by objective.

Objective 1)

Hobo loggers were placed in ten different studs in five different states: IL, MO, NC, NE, TX. Figure 5 presents the daily temperature cycles inside each stud as well as the cooling system used. The ability of each system to effectively moderate the temperature clearly varied among studs. In general results were as expected with cool cells more effective in lower relative humidity environments.

Figure 5. Daily summer temperature cycles inside separate cooperating studs by cooling system.



Objective 2)

Each of the mitigation strategies worked to varying degrees at reducing the impact of elevated ambient temperatures on physiological responses to heat stress. Tables 1 and 2 present least-squares means and standard errors of thee surgically placed Anipill temperature sensors for each treatment period. Above the line are from the Latin Square portion of the trial, and below the line are from the 9 week application of treatment. The mitigation strategies appear to be able to help maintain scrotal temperatures but have little impact on core body temperature. It is likely that coping mechanisms (respiration rate, shunting of blood to the periphery etc.) were able to maintain core body temperature. Figures 6 and 7 present the temperature and respiration rate data by morning and afternoon checks, respectively. These figures demonstrate the effectiveness of the various mitigation strategies at reducing the need for coping mechanisms to maintain core and scrotal temperatures

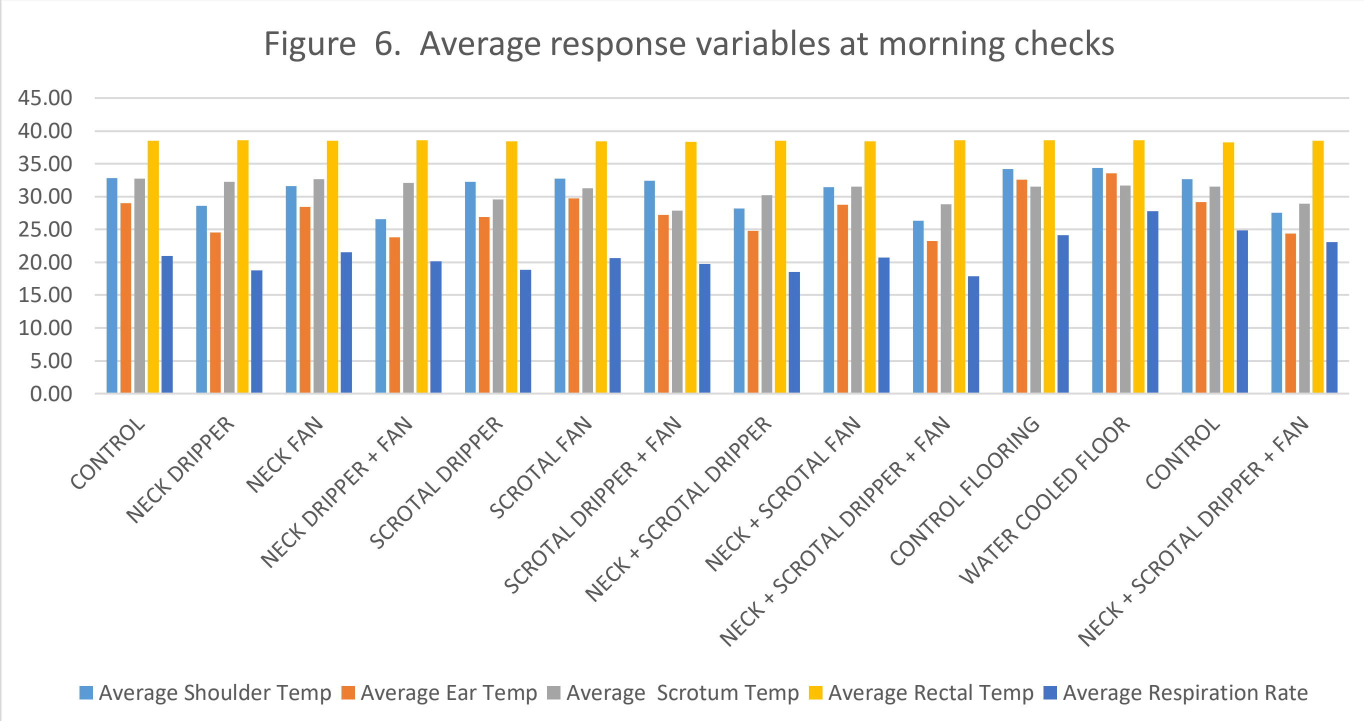
Table 1. Least-squares means and standard errors for abdominal temperature by treatment for all short (above line) and 9 week (below line) periods and number of observations recorded by internal sensor.

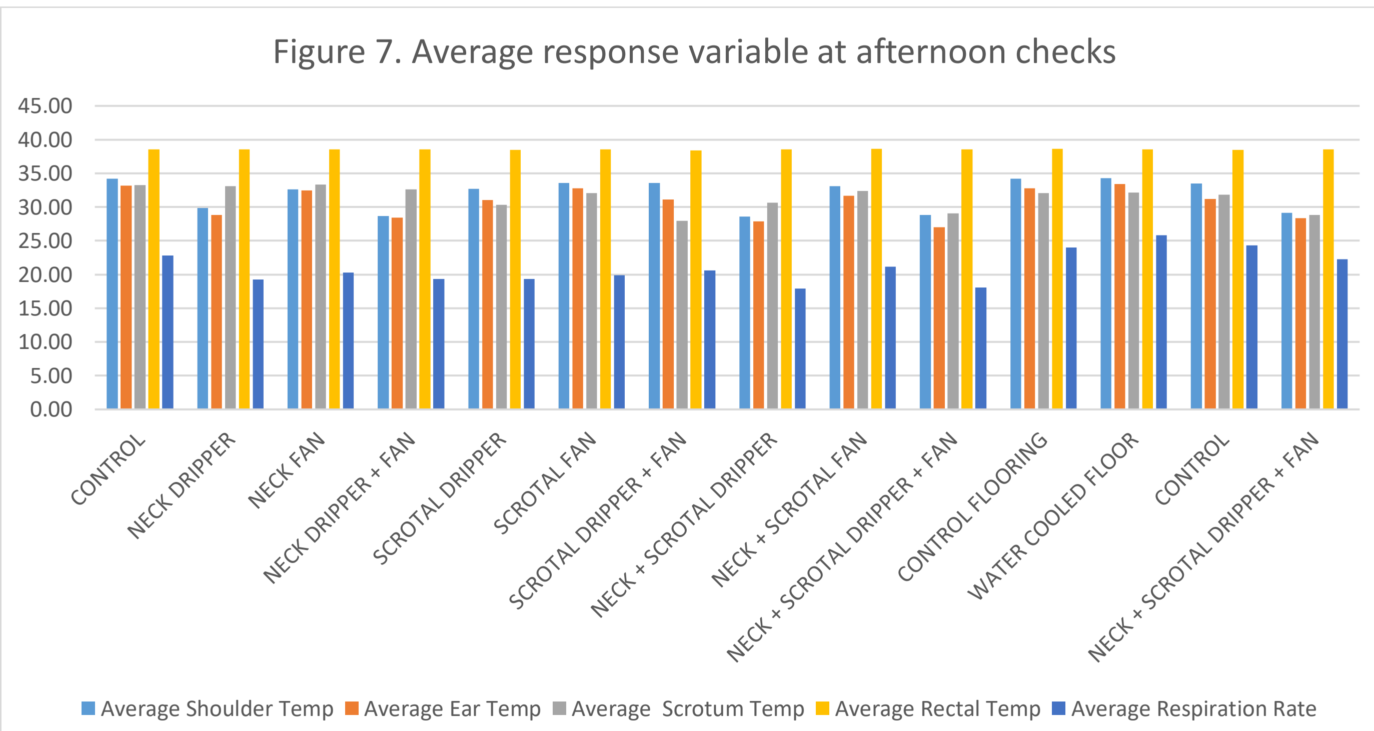
Treatment	TEMP LSMEANS	STANDARD DEVIATION	N
CONTROL	38.97	0.40	1327.00
NECK DRIPPER	38.84	0.32	868.00
NECK FAN	38.87	0.38	1035.00
NECK DRIPPER AND FAN	39.04	0.45	855.00
SCROTAL DRIPPER	38.84	0.34	991.00
SCROTAL FAN	38.97	0.41	1126.00
SCROTAL DRIPPER AND FAN	38.96	0.39	990.00
NECK AND SCROTAL DRIPPER	38.97	0.37	690.00
NECK AND SCROTAL FAN	38.82	0.35	1092.00
NECK AND SCROTAL DRIPPER AND FAN	38.97	0.39	1120.00
CONTROL FLOORING	38.78	0.33	1416.00
CONDUCTIVE COOLING FLOORING	38.90	0.36	1704.00
CONTROL	38.65	0.45	12614.00
NECK AND SCROTAL DRIPPER AND FAN	38.64	0.69	7446.00

Table2. Least-squares means and standard errors for scotal temperature by treatment for all short (above line) and 9 week (below line) periods and number of observations recorded by internal sensor.

Treatment	TEMP LSMEANS	STANDARD DEVIATION	N
CONTROL	34.91	0.85	2414.00
NECK DRIPPER	34.74	0.99	2833.00
NECK FAN	35.01	1.03	2184.00
NECK DRIPPER AND FAN	34.45	0.92	2568.00
SCROTAL DRIPPER	33.36	1.05	2228.00
SCROTAL FAN	34.80	1.07	2397.00
SCROTAL DRIPPER AND FAN	32.74	1.08	2482.00
NECK AND SCROTAL DRIPPER	33.58	0.96	2032.00
NECK AND SCROTAL FAN	34.77	1.21	2491.00
NECK AND SCROTAL DRIPPER AND FAN	33.39	1.36	3109.00
CONTROL FLOORING	34.50	0.84	5986.00
CONDUCTIVE COOLING FLOORING	34.41	0.88	7130.00
CONTROL	33.83	0.94	17509.00

NECK AND SCROTAL DRIPPER AND FAN	33.12	1.14	10810.00





Objective 3)

The treatment most effective at maintaining scrotal temperature and respiration rate was the combination of fans and drippers on both the neck and the scrotum. So all boars were housed under HS conditions, and every other stall had drippers and fans applied to the neck and scrotum of boars. After 9 wk all boars were slaughtered and sperm cells flushed from the epididymis. They were sorted by flow cytometry and are presented below for each individual boar. Targets used by the andrology lab to categorize samples are also included, and comparison shows that the floor cooling was effective at mitigating some of the negative effects of elevated ambient temperatures as well as reducing the variation among individual animals (Table 2).

Table 2. Mitigation effectiveness. Cooled boars had water cooled floor. Flow cytometry was used for gating individual cells into specific classes (those with ubiquitin on the tail, tail abnormality determined by lectin, prosimal cytoplasmic droplets indicated by LCA, intact acrosomes indicated by peanut agglutination assay, and percentage positive for the TUNEL assay indicative of DNA fragmentation, respectively). A target is presented at the bottom.

Trtmnt	**ID**	**%Gated Tail UBB++**	**%Gated Tail LCA++**	**%Gated Proximal CD Present LCA-based**	**%Gated Percent Good Acrosomes PNA-based**	**%Gated TUNEL Positive**
Cooled	3	35.0	16.6	97.8	97.4	0.1
Cooled	4	18.0	2.4	24.6	20.9	1.8
Cooled	7	29.8	1.2	14.4	22.2	0.3
Cooled	8	26.3	15.4	91.8	86.7	3.6
Cooled	11	35.2	2.8	22.6	53.1	0.5
Cooled	12	21.1	2.5	18.5	64.8	0.3
mean		27.6	6.8	45.0	57.5	1.1
Control	1	97.1	69.1	92.6	40.4	0.5
Control	2	33.9	4.2	33.2	17.4	6.1
Control	5	81.6	10.9	59.3	22.3	0.3
Control	6	21.2	4.4	26.7	37.8	0.8
Control	9	66.2	29.6	97.5	82.5	0.1
Control	10	7.0	71.9	95.4	15.8	8.2
mean		51.2	31.7	67.4	36.0	2.7
Target		<30 or 36	<10.0	<25.0	>50	<5

Objective 4)

The response rate from commercial studs was surprisingly poor. We recruited survey completion via e-mail to all stud managers attending the 2016 Boar Stud Managers Conference, personal telephone and e-mail contacts, through companies supplying boar studs and in person at the World Pork Expo. Even so, we received inadequate responses to conduct a meaningful analysis as proposed. While IRB approval has lapsed, if asked for a blank survey it can still be provided and returned versions incorporated into the database for analyses. Funds for the gift cards not used are being returned, so future submissions will be gratis.

Discussion:

The results are fairly self-explanatory. Cooling in any form (fans, drippers, the combination or water cooled floors) helped to mitigate signs of heat stress in boars (i.e. reduced coping mechanisms like respiration rate or skin temperatures). To varying degrees they were also effective at preventing core body temperature from becoming elevated, as indicated by the rectal temperature during mitigation phases. The water cooled floor, which had tap water running under the shoulder portion of boars, was further shown to reduce negative impacts of elevated ambient temperatures on sperm quality traits. The industry should consider the value of mitigation strategies that go beyond cool cells to more effectively maintain zone thermoneutral conditions and perhaps for less cost than air conditioning.