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Analysis of Various Physiological Values of Growing Miniature Pigs in Isolator

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ANALYSISOFVARIOUSPHYSIOLOGICALVALUESOFGROWINGMINIATUREPIGSINISOLATOR

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Young Ah Lee [α] & Jin Soo Han [σ]

Abstract- The objective of this study was to provide physiological values on the growing miniature pigs in isolator. Measurements were taken of each miniature pigs' body weight, temperature, hematological and serum biochemistries, and heart rate. The miniature pigs were divided into 2 groups. The piglet group (n = 8), consisting of animals approximately 4 weeks old and weighing 5 to 7 kg, were used to obtain normal physiological values at 6, 10, 14, 18, and 22 weeks of age. The adult group (n = 8), which consisted of animals approximately 40 weeks old weighing 19 to 21.3 kg, was used to obtain normal physiological values for miniature pigs at 43, 47, 51, 55, and 59 weeks of age. None of the animals displayed abnormal behavior or symptoms while they were bred in the isolators. The weight of every animal that was bred in an isolator gradually increased. Among the center of the animals' foreheads, necks, lateral abdominal areas, and hips, the lateral abdominal areas are showed the highest temperature variation in all tests as determined using an infrared thermometer. The author confirmed that all of the results from the complete blood cell counts (CBCs), serum biochemical tests, and heart rates were within the normal range for miniature pigs.

This study provides a baseline for interpreting physiological data for miniature pigs growing within isolator systems. We expect our study to help other researchers studying miniature pigs and to make a significant contribution in the field of bio-organ transplantation.

Keywords: isolator, miniature pigs, physiological values.

I. Introduction

Pigs have become widely used for biomedical research in recent years since there are many similarities in metabolic and cardiovascular function between swine and humans (Swindle *et al.*, 1988). Miniature pigs specifically make good research animals, since they are easy to deliver, maintain, and utilize in isolators (Mandel and Travnicek, 1987). Their small size relative to that of other large animals facilitates housing and handling, while their ample blood volume allows for more frequent serial blood collections than are possible with rodents. Furthermore, miniature pigs have received attention as potential donors in xenotransplantation because of their ability to be bred in an aseptic environment, their high fertility, and low costs (Park *et al.*, 2006). Isolator systems are used to maintain laboratory and farm animals in a sterile environment to prevent contact-transmitted and airborne infections (Trexler, 1973). While isolators have been largely used in general laboratory animal research, most of the literature contains information pertaining to rodents, and there is little available information regarding optimal isolator design and conditions for miniature pigs.

The establishment of standard basic data is important in the biosciences, because it can reduce and refine the use of laboratory animals (Michael *et al.*, 2006). Although there have been studies to acquire basic data regarding the use of miniature pigs, no data is available regarding their use within an isolator system. Therefore, the aim of this study, in addition to identifying baseline physiological values for miniature pigs growing within an isolator, was to identify optimal isolator designs and conditions for miniature pigs.

II. Materials and Methods

a) *Advanced isolator*

The miniature pigs were housed in groups of 2 in isolators (1200W × 900D × 950H m/m, SK-ISO-1700HBP600, Three-shine INC, Daejeon, Korea, Fig. 1). The internal temperatures of the isolators were set to 23 ± 2°C, and their relative humidity was kept at 50 ± 10%. The ventilation frequency was 50 times per h, illuminance was 100–150 Lux, and noise levels were kept below 58 phon, when possible. Feeding units and water nozzles were installed in the isolators, which consisted of space for breeding and movable room-dividing sub-frames set on wheels. The sub-frames were equipped with a window for easy animal observation, and gloves for gnotobiotic handling of the animals. Gloves were protected from damage within the isolator by drawing them back into the glove housing after use, and a double-capped transport chamber was used to sterilize the goods coming in and out of the isolator. In short, the inner part of the cap was airtight, so that the outer cap could be removed to either add goods for sterilization and insertion, or extraction of sterilized waste.

The feed unit was securely fixed within the cage, and could be easily washed through a hole in its bottom. Automated watering valves were used to allow *ad libitum* consumption, and a cleaning box containing a water gun was attached for easy cleaning of the isolator's interiors. An uninterruptible power supply was installed to retain operation of the exhaust port in case

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of a blackout, ventilating the isolator via a safety filter to protect against the culling of miniature pigs mid-experiment. The drain under the isolator was installed to make the excrement of the pigs fall downward, and the sub-drain switch valve can be used to remove the body wastes selectively.

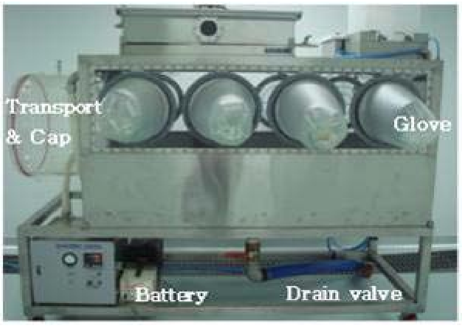

Figure 1: Isolator system for miniature pigs

This system is composed of a stainless steel body containing an entry port, gloves, control box, battery, drain valve, filter boxes, and other variable equipment.

b) Animals

Specific pathogen-free miniature pigs (PWG Micropig, mixed with Yucatan, native pigs, pygmy pigs, and miniature pot-bellied pigs) were obtained from Medi Kinetics Co. (Pyeongtaek, Korea). All of the pigs were quarantined for 4 weeks before use in experiments, and were clinically healthy prior to the study. Miniature pigs were divided into 2 groups. The piglet group (n = 8), consisting of animals approximately 4 weeks old and weighing 5 to 7 kg, were used to obtain normal physiological values at 6, 10, 14, 18, and 22 weeks of age. The adult group (n = 8), which consisted of animals approximately 40 weeks old which weighed 19 to 21.3 kg, was used to obtain normal physiological values for miniature pigs at 43, 47, 51, 55, and 59 weeks of age.

c) Clinical symptoms

The health status of each miniature pig was monitored daily throughout the duration of the study. In case any abnormality was detected, a description of the severity and frequency of the symptoms was recorded by date.

d) Measurements of body weight and body temperature

The miniature pigs were weighed using a balance (HBS-510L; CAS, Korea). We measured the body surface temperature of the miniature pigs using a non-contact infrared thermometer, which allowed for measurement of their body temperatures without causing them any undue stress. Temperatures were measured on the center of the forehead, neck, lateral abdominal area and hip (eye exposure to the beam was avoided).

e) Hematological and serum biochemistry analysis

All pigs were anesthetized prior to blood collection using medetomidine 0.2 mg/kg (Domitor®; Pfizer Korea, Seoul, Korea) intramuscularly and tiletamine–zolazepam 4.4 mg/kg (Zoletil®; Virbac, Carros, France) intramuscularly. All miniature pigs were bled (5 mL) through their jugular veins. All blood samples were analyzed for evaluated using an automated hematology analyzer (FORCYTE; Oxford Science, USA) for CBC and differential blood cell counts. In addition to the red blood cell (RBC), hemoglobin concentration (Hb), and platelet (PLT) counts we did, we also quantified the content of several different cell populations within the white blood cell count (WBC), including: neutrophils (NEU), lymphocytes (LYM), monocytes (MONO), eosinophils (EOS) and basophils (BASO).

All serum samples were analyzed for complete total protein (TP), glucose (GLU), uric acid (URIC), calcium (CA), cholesterol (CHOL), total bilirubin (TBIL), creatine (CREA), alanine aminotransferase (ALT), albumin (ALB), aspatate aminotransferase (AST), and cortisol levels. All serum analysis was measured using a radioimmunoassay (RIA; PerkinElme, Finland) in the clinical laboratory of the Neodin Medical Institute (Seoul, Korea). The mean and SE were calculated for each of the measured parameters. Reference values for the hematology of pigs are documented well in previous literature.

f) Heart rate measurements

Heart rates of the 6- and 43-week-old animals were monitored and recorded. All pigs were anesthetized using medetomidine 0.2 mg/kg (Domitor®; Pfizer Korea, Seoul, Korea) intramuscularly and tiletamine–zolazepam 4.4 mg/kg (Zoletil®; Virbac, Carros, France) intramuscularly prior to collection of their heart rate data. During the 24 h of monitoring, we cleaned the isolators and fed the pigs while they were attached to the holter monitor for examination of their heart rates during their typical daily activities within the isolators. After 24 h, we anesthetized the animals once again, removed the monitors and then compiled the data.

g) Statistical analysis

All statistical analyses were performed using Graph Pad Prism version 4.0 for Windows (Graph Pad Software, San Diego, CA, USA). Data were recorded as mean ± standard error of the mean (SEM).

Comparisons of the two different groups were made by Student's unpaired *t*-tests and P values$<$0.05 were considered significant.

III. Results

a) Clinical symptoms

All animals showed no clinical signs of disease and appeared healthy throughout the study.

b) Measurements of body weight and body temperature

The recorded body weight measurements are shown in Table 1. The weight of every animal that was bred in an isolator gradually increased. The results of the body temperature measurements are shown in Figures 2 and 3. The center of the forehead, neck, lateral abdominal area, and hip showed the highest temperature variations in all tests. The lowest temperature values occurred in the forehead region for all miniature pigs, and were lower in the 55-week-old miniature pigs (33.0°C) compared to other adult groups. The temperature of the abdominal region was higher in the 6-week-old miniature pigs (35.93°C) than in the other groups, and abdominal skin temperature values were considered to be very to the rectal temperatures obtained of miniature pigs by using an infrared thermometer.

c) Hematological and serum biochemistry analysis

Hematological analysis results are shown in Tables 2 and 3. Total WBCs, neutrophil, and lymphocyte counts of the piglet group tended to decrease with age ($P < 0.05$). The total WBCs and neutrophil counts of the adult group tended to decrease with age, although data was not statistically significant between the age groups. The results of the serum biochemistry analysis are shown in Tables 4 and 5. Cortisol levels of the piglet group decreased with age. The cortisol values for the 6–week-old miniature pigs (18.04 ± 3.18 ug/dL) were higher than those for all other age groups. Although cortisol values of the piglet group tended to be greater than those of the adult group, the differences were not statistically significant. Serum biochemistry values did not differ among the age groups and were not statistically significant between the age groups. All hematological and serum biochemistry values were within the normal reference range.

d) Heart rate measurements

The results of heart rate measurements are shown in Table 6 and Fig. 4. The heart rates of minimum, maximum, and average in 6-week-old animals were 84.17 ± 10.39, 186.55 ± 23.96, and 121.89 ± 16.77, respectively. The minimum, maximum, and average heart rates of 43-week-old animals were 68.57 ± 9.92, 190.96 ± 10.73, and 111.69 ± 12.93, respectively. It should be noted that the isolator pigs' average heart rates increased above a normal heart reference rate (135) during cleaning and feeding periods (data not shown).

Table 1: Body weight changes of miniature pigs within the isolator systems

Group	Age (weeks)	Body weight (kg)
Piglet	6	6.06 ± 0.67
	10	7.94 ± 0.57
	14	9.35 ± 1.31
	18	10.89 ± 2.48
	22	12.24 ± 3.18
Adult	43	20.24 ± 0.67
	47	21.68 ± 1.70
	51	23.56 ± 1.95
	55	25.78 ± 2.69
	59	27.40 ± 1.68

Values are mean ± SE (n = 8).

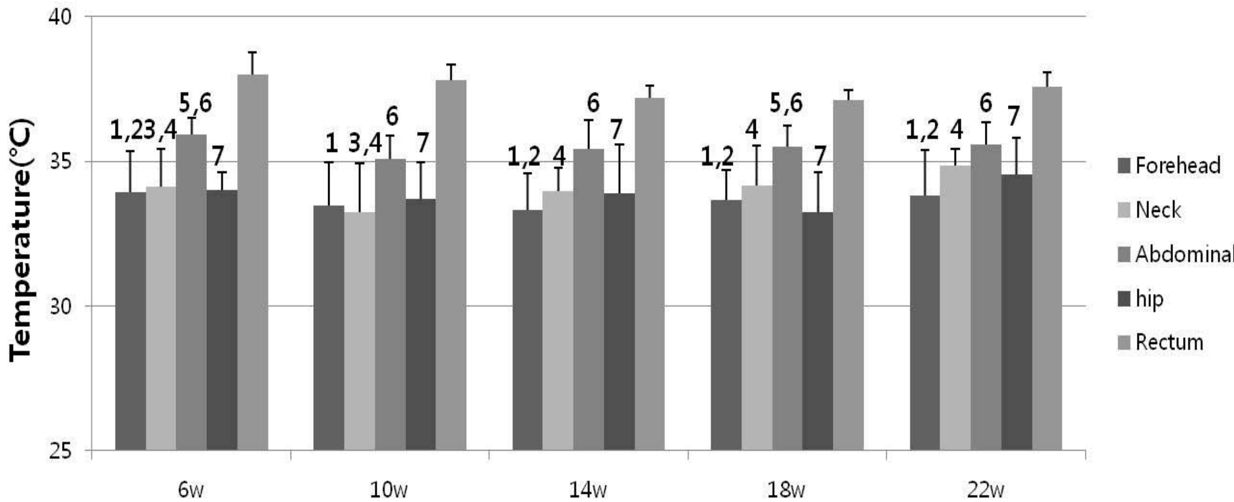

Figure 2: Comparison of body temperatures (℃) from the piglet group within the isolator systems

Bars represent the mean values and standard errors of body temperature (n = 8). One-way analysis of variance (ANOVA) tests revealed significant differences in 5 regions ($P < 0.05$); [1]Significant difference between the forehead and abdomen; [2]Significant difference between the forehead and rectum; [3]Significant difference between the neck and abdomen; [4]Significant difference between the neck and rectum; [5]Significant difference between the abdomen and hip; [6]Significant difference between the abdomen and rectum; [7]Significant difference between the hip and rectum.

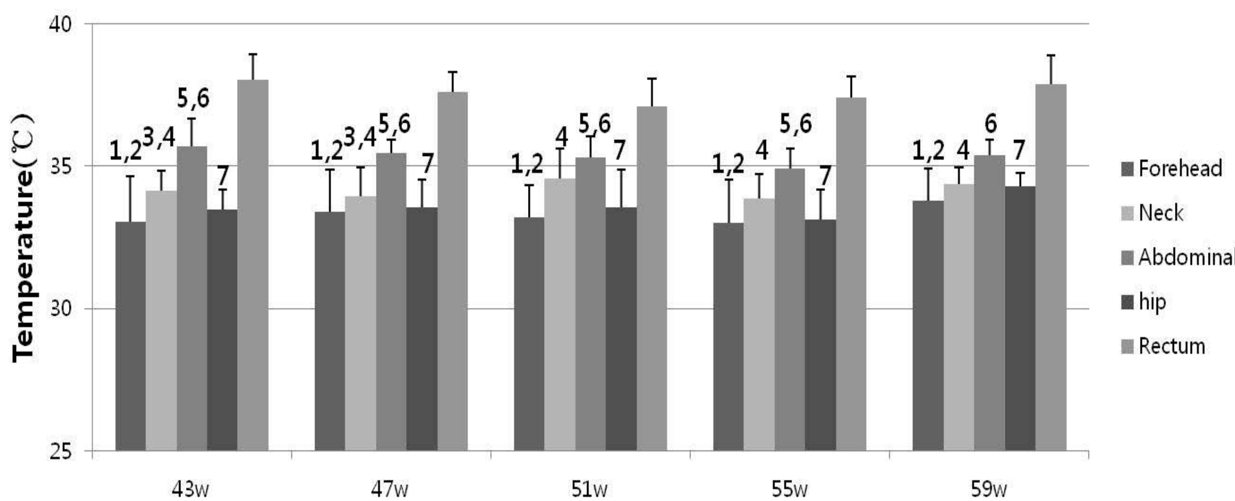

Figure 3: Comparison of body temperatures (℃) from the adult group within the isolator systems

Bars represent the mean values and standard errors of body temperature (n = 8). One-way analysis of variance (ANOVA) tests revealed significant differences in 5 regions ($P < 0.05$); [1]Significant difference between the forehead and abdomen; [2]Significant difference between the forehead and rectum; [3]Significant difference between the neck and abdomen; [4]Significant difference between the neck and rectum; [5]Significant difference between the abdomen and hip; [6]Significant difference between the abdomen and rectum; [7]Significant difference between the hip and rectum.

Table 2: Hematological values from the piglet group within the isolator systems

	Normal Range	6 weeks	10 weeks	14 weeks	18 weeks	22 weeks
White blood cells (K/uL)[1]	4.4-26.4	20.3 ± 6.55[1]	14.28 ± 14.28	12.48 ± 4.50	10.94 ± 2.94	10.18 ± 3.54
Neutrophils (K/uL) [1]	3.1-11.2	9.87 ± 5.95	5.07 ± 5.07	4.96 ± 1.70	4.98 ± 1.56	4.27 ± 1.66
Lymphocytes (K/uL) [1]	4.3-13.6	8.85 ± 2.57	7.52 ± 7.52	6.31 ± 2.99	5.22 ± 1.39	4.84 ± 1.39
Monocytes (K/uL)	0.2-2.2	0.69 ± 0.36	0.69 ± 0.69	0.52 ± 0.15	0.36 ± 0.21	0.52 ± 0.37
Eosinophils (K/uL)	0.1-2.4	0.83 ± 0.74	0.98 ± 0.98	0.69 ± 0.82	0.35 ± 0.19	0.51 ± 0.76
Basophils (K/uL)	0.0-0.4	0.06 ± 0.03	0.04 ± 0.04	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.03
Red blood cells (M/uL)	5.3-9.25	6.24 ± 1.51	6.69 ± 6.69	7.21 ± 1.00	5.73 ± 0.66	5.65 ± 1.38
Hemoglobin (g/dL)	9.0-15.8	9.51 ± 2.18	10.25 ± 10.25	10.53 ± 1.42	8.87 ± 1.00	8.73 ± 2.28
Platelets (K/uL)	148-898	374.06 ± 203.9	343.75 ± 343.8	370.13 ± 151.7	336.13 ± 90.0	289.00 ± 148.6

Values are mean ± SE (n = 8). [1] Indicates that the parameter decreased significantly with increasing age ($P < 0.05$). Normal range (Bollen *et al*., 2000; Swindle *et al*., 2007).

Table 3: Hematological values from the adult group within isolator systems

	Normal Range	43 weeks	47 weeks	51 weeks	55 weeks	59 weeks
White blood cells (K/uL) [1]	4.4-26.4	11.78 ± 3.95	11.23 ± 3.57	10.21 ± 1.39	10.11 ± 2.74	9.51 ± 3.17
Neutrophils (K/uL) [1]	3.1-11.2	5.22 ± 2.01	4.82 ± 1.67	4.27 ± 1.12	4.03 ± 1.46	3.69 ± 1.53
Lymphocytes (K/uL)	4.3-13.6	5.46 ± 2.18	5.03 ± 2.45	5.21 ± 1.41	5.45 ± 1.17	4.97 ± 1.59
Monocytes (K/uL)	0.2-2.2	0.43 ± 0.23	0.56 ± 0.2	0.41 ± 0.20	0.38 ± 0.18	0.45 ± 0.21
Eosinophils (K/uL)	0.1-2.4	0.62 ± 0.52	0.79 ± 1.19	0.29 ± 0.28	0.24 ± 0.22	0.36 ± 0.45
Basophils (K/uL)	0.0-0.4	0.04 ± 0.03	0.04 ± 0.03	0.03 ± 0.04	0.02 ± 0.02	0.04 ± 0.02
Red blood cells (M/uL)	5.3-9.25	6.43 ± 0.57	6.80 ± 0.83	6.70 ± 1.61	6.29 ± 0.82	6.77 ± 1.86
Hemoglobin (g/dL)	9.0-15.8	9.91 ± 1.87	11.16 ± 1.88	10.42 ± 2.88	10.28 ± 1.72	10.46 ± 2.64
Platelets (K/uL)	148-898	391.9 ± 99.5	311.9 ± 90.7	284.2 ± 110.2	339.6 ± 94.2	323.8 ± 92.3

Values are mean ± SE (n = 8). [1] Indicates that the parameter decreased with increasing age. Normal range (Bollen *et al*., 2000; Swindle *et al*., 2007).

Table 4: Serum biochemistry values from the piglet group within the isolator systems

	Normal Range	6 weeks	10 weeks	14 weeks	18 weeks	22 weeks
Total Protein(g/dL)	2.25 - 8.0	5.08 ± 1.75	4.93 ± 1.97	4.88 ± 1.37	4.74 ± 1.48	5.73 ± 1.25
ALB (g/dL)	1.8 - 3.3	3.23 ± 0.83	3.06 ± 0.77	3.00 ± 0.37	2.89 ± 0.73	3.19 ± 0.52
CREA (mg/dL)	0.5 - 2.1	0.59 ± 0.26	0.63 ± 0.3	0.71 ± 0.27	0.71 ± 0.35	0.85 ± 0.22
URIC (mg/dL)		0.47 ± 0.34	0.38 ± 0.38	0.45 ± 0.32	0.38 ± 0.38	0.37 ± 0.4
Glucose (mg/dL)	43 - 133	112.13 ± 36.05	91.13 ± 37.4	82.38 ± 19.1	72.13 ± 21.24	74.75 ± 20.19
TBIL (mg/dL)	0.0 - 0.3	0.08 ± 0.05	0.08 ± 0.05	0.08 ± 0.05	0.08 ± 0.05	0.08 ± 0.04
AST (IU/L)	16 - 65	55.88 ± 22.42	46.50 ± 17.77	48.63 ± 27.37	41.00 ± 19.15	43.13 ± 17.82
ALT (IU/L)	9 - 43	37.88 ± 11.53	35.00 ± 10.2	38.50 ± 17.57	32.13 ± 16.69	40.00 ± 16.24
CHOL (mg/dL)	18 - 79	47.25 ± 14.82	44.25 ± 21.61	48.50 ± 18.59	41.25 ± 18.82	54.13 ± 15.49
Calcium (mg/dL)	6.5 - 11.4	9.98 ± 1.92	8.70 ± 2.48	8.55 ± 1.41	7.98 ± 2.01	8.95 ± 1.54
Cortisol (ug/dL)		18.04 ± 3.18	4.32 ± 3.81	12.01 ± 5.13	10.43 ± 5.03	9.85 ± 5.73

Values are mean ± SE (n = 8). ALB (albumin), CREA (creatine), URIC (uric acid), TBIL (total bilirubin), AST (aspatate aminotransferase), ALT (alanine aminotransferase), CHOL (cholesterol). Normal range (Bollen *et al*., 2000; Swindle, 2007).

Table 5: Serum biochemistry values from the adult group within the isolator systems

	Normal Range	43 weeks	47 weeks	51 weeks	55 weeks	59 weeks
Total Protein (g/dL)	2.25 - 8.0	6.16 ± 2.23	5.73 ± 2.21	6.45 ± 1.62	6.19 ± 2.04	6.75 ± 1.75
ALB (g/dL)	1.8 - 3.3	2.93 ± 1.02	2.81 ± 1.00	3.31 ± 0.62	3.14 ± 0.8	3.18 ± 0.47
CREA (mg/dL)	0.5 - 2.1	0.69 ± 0.37	0.74 ± 0.32	0.9 ± 0.25	0.97 ± 0.28	0.95 ± 0.1
URIC (mg/dL)		0.38 ± 0.38	0.47 ± 0.36	0.33 ± 0.38	0.35 ± 0.37	0.3 ± 0.37
Glucose (mg/dL)	43 - 133	91.25 ± 55.74	69.38 ± 42.39	90.88 ± 39.44	79.38 ± 45.06	89.13 ± 46.56
TBIL (mg/dL)	0.0 - 0.3	0.09 ± 0.06	0.18 ± 0.3	0.08 ± 0.05	0.1 ± 0.05	0.13 ± 0.05
AST (IU/L)	16 - 65	60.5 ± 28.25	48.88 ± 19.05	39.38 ± 12.98	34.75 ± 14.95	48.38 ± 25.65
ALT (IU/L)	9 - 43	27.38 ± 7.93	30.50 ± 13.85	28.63 ± 6.46	26.63 ± 10.51	31.25 ± 14.06
CHOL (mg/dL)	18 - 79	43.63 ± 21.79	46.25 ± 20.42	47.75 ± 23.56	48.25 ± 20.73	54.25 ± 24.21
Calcium (mg/dL)	6.5 - 11.4	8.98 ± 2.34	8.16 ± 2.37	9.08 ± 1.6	8.86 ± 2.31	9.41 ± 1.8
Cortisol (ug/dL)		9.99 ± 2.33	9.79 ± 3.93	8.2 ± 3.03	9.85 ± 2.81	9.74 ± 6.41

Values are mean ± SE (n = 8). ALB (albumin), CREA (creatine), URIC (uric acid), TBIL (total bilirubin), AST (aspatate aminotransferase), ALT (alanine aminotransferase), CHOL (cholesterol). Normal range (Bollen *et al*., 2000; Swindle, 2007).

Table 6: Heart rate values of miniature pigs within the isolator systems

Age	6 weeks	43 weeks
Minimum	84.2 ± 10.4	68.6 ± 9.9
Average	121.9 ± 16.8*	111.7 ± 12.9
Maximum	186.6 ± 24.0	191.0 ± 10.7

Values are mean ± SE (n = 8). *Significant ($P < 0.05$) differences between values for 6 and 43 weeks. (Student's unpaired *t*-test, two-tailed)

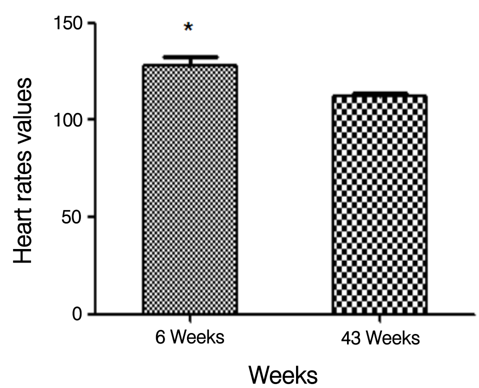

Figure 4: Heart rates of miniature pigs within the isolator systems

Bars represent the mean values and standard errors of the pigs' heart rate values. *Significant ($P < 0.05$) difference between values at 6 and 43 weeks. (Student's unpaired *t* –test, two-tailed).

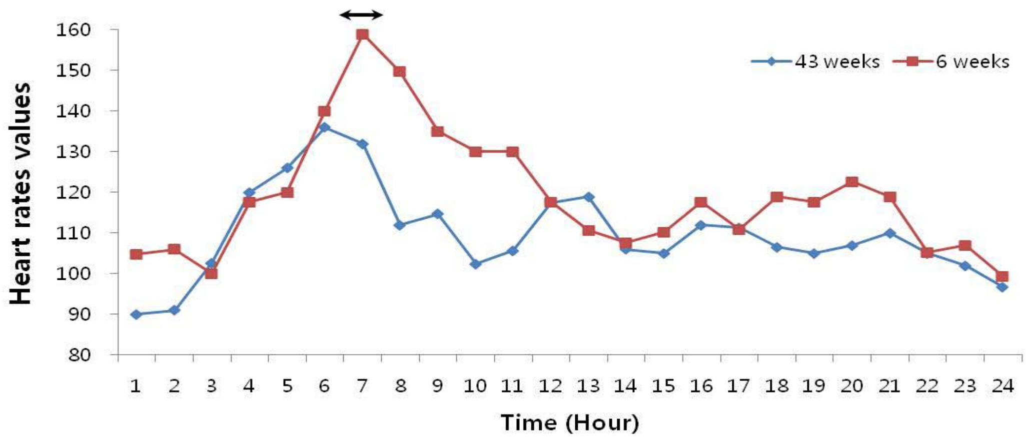

Figure 5: Heart rate trends of miniature pigs within the isolator systems

Hourly heart rates over a 24-h period. The arrow indicates the period during cleaning of the isolator, at which time the heart rate increased immediately.

IV. Discussion

Pigs are exposed to many stress factors that may affect their health and welfare, including housing and management (Ruis *et al*., 1997). Typical signs of stress in pigs include depressed activity levels and decreased feed intake, which typically result in weight loss (Seymour *et al*., 1964; McGlone *et al*., 1987; Christon, 1988). None of the animals used in this study displayed abnormal behaviors or symptoms while they were bred in the isolators, and body weight measurements of all of the animals showed gradual body weight gains.

Temperature measurement is basic for medical evaluation, and is a tool that is frequently used in both clinical and research settings (Quimby *et al*., 2009). Body temperature is most commonly collected rectally in clinical settings, because it is an accurate reflection of core body temperature (Greer *et al*., 2007). This method is very difficult to utilize for animals housed within isolator environments, however, therefore we used a non-contact infrared thermometer. Since non-contact infrared thermometers typically require 2–4°C of calibration, the results should be considered when selecting a particular temperature region for experimental use with a non-contact infrared thermometer in miniature pigs. It is important to note that noninvasive temperature-measurement techniques that are rapidly accomplished could contribute to laboratory animal stress reduction and improved welfare (Chen *et al*., 2006). Results indicated that the areas exhibiting the highest temperatures in all tests were lateral abdominal area.

In order to measure the stress of breeding within an isolator, we monitored the cortisol levels in the animals' blood serum as well as the variation in their heart rates via a holter monitor. There was no significant difference between the average heart rates of 6- and 43-week-old animals. Although heart rates abruptly increased when there were workers outside of the isolators to clean or provide feed, this is likely due in response to cleaning noises and expectations of being fed. Additionally, while gradual increases and decreases in heart rates were observed during the animals' dark-cycle sleeping hours, they were not regarded as abnormal symptoms of stress because reports indicate that heart rate also varies in humans during sleep (Bonnet and Arand, 1997).

Increased cortisol level is an important indicator of stress (Carroll *et al*., 2006), and serum concentrations have been widely used to assess the effects of different stressors on immune function (Bilandzić *et al*., 2006). Cortisol is known to be the primary glucocorticoid released during times of stress in pigs (Kojima *et al.*, 2007), and administration of morphine and fentanyl during surgical procedures have been shown to decrease postoperative cortisol concentrations relative to controls in swine weighing 20 kg (Malavasi *et al*., 2006). The results indicate that cortisol levels were elevated in both the piglet and adult groups. Since transportation, weaning, and maternal separation have been shown to increase cortisol concentrations in pigs (Cooper *et al*., 2009; Kojima *et al*., 2008; Nyberg *et al*., 1988; Parrot and Mission., 1989), the author attribute transportation stress to the increased cortisol levels in the 6- and 43-week old animals. Since cortisol levels were the highest in the 6-week-old animals, we assume that their young age caused them to be more susceptible to stress. The data agrees with previous studies, which indicated that increased stress resulted in elevation of WBC numbers and cortisol concentration (Morrow-Tesch *et al*., 1994; Kojima *et al*., 2009). These elevated WBC and cortisol concentrations decreased with increasing age in all of this study's miniature pigs. The author confirmed that all of the results from the CBC and serum biochemical tests were within the normal range. The data presented in this study provide a baseline for interpreting physiologic results of data gathered during the growth of miniature pigs within isolator systems.

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