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蘭嶼豬公豬於高溫環境下之產精性狀表現 (1)

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摘　要

公豬繁殖性狀取決於動物本身之內在因子如年齡、品種與營養，以及外在環境因素如動物照護與畜舍條件等因素。雖不同品種公豬的精液對於溫度變化的敏感度不同，一般而言精液品質與環境溫度變化仍息息相關。為瞭解高溫環境對蘭嶼豬公豬繁殖性狀之影響，本試驗將其飼養於常溫 (25 － 30℃) －溫濕度指數 (temperature humidity index, THI) 平均 81 與高溫 (30 － 35℃) － THI 平均 90 之豬欄 8 週，每 2 週自附睪採精一次，並以電腦輔助精子分析儀 (computer-assisted sperm analysis) 分析精子性狀。結果顯示，經高溫環境 (高度熱緊迫環境) 處理 6 週後，精子存活率已降至 69.5 ± 6.2%；處理 8 週後精子存活率、前進式活力 (progressive motility, PR) 與精子活力，分別降至 62.6 ± 7.6、54.3 ± 7.0 與 67.3 ± 6.8%，皆顯著低於常溫組 (中度熱緊迫環境) (83.4 ± 1.8、84.3 ± 0.9 與 89.2 ± 1.8%)。試驗第 8 週與第 0 週相比，高溫組之精子存活率、前進式活力與精子活力，分別平均下降 25.1、24.1 與 23.6%。經 8 週高溫環境處理後，睪丸長度為 7.0 ± 0.2 cm 比常溫組的 7.8 ± 0.3 cm 顯著萎縮約 10%，組織切片結果顯示，其生精小管 (seminiferous tubule) 亦出現萎縮及空泡化，顯示高溫環境已破壞其造精能力。因此，蘭嶼豬公豬長期處於高溫環境會造成睪丸萎縮與生精小管之萎縮及空泡化，影響其造精能力，並使精子死亡率提高與精子活力減少。

關鍵詞：蘭嶼豬、高溫環境、產精性狀、精子、睪丸。

緒　言

公豬精液品質取決於動物本身之內在因子如年齡、品種與生理狀況，以及外在環境因素如動物照護與畜舍條件等因素 (Gorski *et al.*, 2017)。臺灣位於亞熱帶地區，高溫多濕的環境對公豬精液品質、產出量與繁殖能力有不良影響，如藍瑞斯與約克夏公豬之精液對高溫敏感，夏季時精液品質較冬季差 (郭等，1997)。由於公豬精子的蛋白質品質攸關生精作用、精子活力與受精能力，長期的夏季高溫易使精子蛋白質流失，因此公豬繁殖力於夏季高溫時普遍較冬季低溫時差 (Martín-Hidalgo *et al.*, 2020)，然而若暴露於高溫環境時間不長，經適當處理後公豬仍有機會恢復正常繁殖能力 (Wettemann and Bazer, 1985)。除了環境溫度的影響外，精液之稀釋或儲存溫度亦會影響精液品質 (Vyt *et al.*, 2004; Lopez Rodriguez *et al.*, 2012)。因此，無論是前端在動物體內生產時的環境溫度，或是後端在精液收集處理時的溫度變化，皆會顯著影響精液品質。

蘭嶼豬 (Lanyu pigs) 為臺灣本土的小型豬，經行政院農業委員會畜產試驗所臺東種畜繁殖場於 1980 年引進選育後，已逐步推廣做為國內生醫用小型豬，目前已應用於心血管疾病 (Liu *et al.*, 2015; Li *et al.*, 2017)、組織工程 (Chang *et al.*, 2016; Hsieh *et al.*, 2018) 與幹細胞治療研究 (Yang *et al.*, 2013; Liao *et al.*, 2018) 等領域。此外，相較於國外的哥廷根小型豬 (Göttingen minipigs)，蘭嶼豬的血液生理值與人類較相近 (吳及章，2018)，適合做為人類臨床前生醫研究的試驗動物。為瞭解高溫環境對國內本土生醫用蘭嶼豬公豬繁殖性狀之影響，本試驗將其飼養於 30 － 35℃之高溫豬欄，定期採精並以電腦輔助精子分析儀 (computer-assisted sperm analysis, CASA; Microptic S. L., Spain) 分析高環境溫度下之精子性狀，以評估蘭嶼豬公豬長期處於高溫環境下，對於其精子性狀與產精能力之影響。

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材料與方法

I. 試驗豬隻

試驗選用 9 隻 6 月齡性成熟蘭嶼豬公豬 (Lanyu 200)，係購自行政院農業委員會畜產試驗所臺東種畜繁殖場，試驗之進行遵照行政院農業委員會畜產試驗所實驗動物照護及使用委員會 (Institutional Animal Care and Use Committee) 審定之規定 (LRI IACUC, No. 109-04)。豬隻於豬舍適應 1 週後，隨機獨立飼養於常溫 (normal temperature treatment) (豬欄內實際溫度，25 － 30℃) (N = 4) 與高溫 (high temperature treatment) (30 － 35℃) (N = 5) 之高床欄位，飼料與飲水皆採任食。豬欄結構以鍍鋅鋼管為主體，常溫組不做任何變化；高溫組則以約 2 cm 厚之合板包覆豬欄四周圍欄以保持豬欄溫度，並於豬欄內部前後兩端離床面約 100 cm 處架設兩盞 250 W 的紅外線保溫燈，使豬欄內溫度維持於 30 至 35℃間。豬欄備有溫濕度計，記錄每天豬欄內的溫濕度變化，持續 8 週。將測得之溫濕度轉換成溫濕度指數 (temperature humidity index, THI) 以評估蘭嶼豬公豬熱緊迫程度，計算公式如下 (NWSCR, 1976)：

$$THI = [(1.8 \times T) + 32)] - [0.55 \times (RH / 100)] \times [(1.8 \times T) + 32 - 58]$$

T 為空氣溫度 (℃)，RH 為相對濕度 (%)。

II. 精液採集

因蘭嶼豬野性較強，不易於假母臺上採精，因此改以麻醉後由附睪採精。公豬分組後，每 2 週進行附睪尾採集精液 1 次。公豬以肌肉注射 8 mg/kg 劑量之 Zoletil® 50 (Virbac corporation, Carros, France) 麻醉後，酒精消毒附睪處之陰囊表皮。以手固定附睪後，將 1 mL 針筒搭配 24 G 針頭直接將針尖刺入附睪尾，採集約 100 μL 精液進行精子性狀分析。

III. 精子性狀分析

採集之精液以 CASA 分析精子性狀，並參考 Broekhuijse *et al.* (2012) 所述參數，進行蘭嶼豬公豬精子活動能力分析 (kinematic analysis)。上機前將精子濃度以 Beltsville Thawing Solution (BTS，每公升含 37.0 g glucose、1.25 g EDTA、6.0 g sodium citrate、1.25 g sodium bicarbonate 與 0.75 g potassium chloride) (Johnson *et al.*, 2000) 調整為 3×10^7 個精子 /mL，取 3 μL 置於四室計數玻片 (Leja, B.V. Nieuw Vennep, Netherlands)，使每個視野下之精子數量介於 80 至 120 個，每個樣品攝取 5 張影像，並以電腦分析精子活力 (即可動精子比率，motility; %)、前進式活力 (progressive motility, PR; %) (即精子向前移動之比率)、曲線移動速率 (curvilinear velocity, VCL; μm/s)、直線移動速率 (velocity straight line, VSL; μm/s)、平均移動路徑 (velocity average path, VAP; μm/s)、直線前進之比率 (linearity, LIN; VSL/VCL; %)、直線趨勢 (straightness, STR; VSL/VAP; %)、擺動性 (wobble, WOB; VAP/VCL; %)、精子平均側擺幅值 (lateral head displacement, ALH; μm)，以及精子頭部擺動與平均路徑交叉之頻率 (beat cross frequency, BCF; Hz) 等運動參數 (圖 1)，每個樣品重複檢測 3 次。

精子存活率 (viability) 以 Hoechst 33342 和 propidium iodide(PI) 螢光染色劑進行染色分析。將精子濃度以 BTS 調整為 3×10^7 個精子 /mL，取 2 μL 的 Hoechst 33342 與 98 μL 的精液混合，於 37℃培養箱靜置 5 分鐘。接著取上述混合液 20 μL 與 2 μL 的 PI 混合，於 37℃培養箱靜置 5 分鐘。最後取 5 μL 樣品置於玻片上，以倒立螢光顯微鏡檢驗精子存活狀況，再以 CASA 分析死活精子數量比率，以百分比表示精子存活率。

VI. 睪丸組織切片分析

試驗豬隻於為期 8 週的試驗結束後，先以肌肉注射 8 mg/kg 劑量之 Zoletil® 50 麻醉，再以 2 － 2.5% 的 isoflurane (Baxter, Guayama, Puerto Rico) 氣體維持全身麻醉，以手術方式摘取雙邊睪丸。睪丸經磷酸鹽緩衝生理食鹽水 (phosphate buffered saline) 清洗後，浸泡於 10% 中性福馬林固定 1 週，測量睪丸長度後，再將樣品送至國立中興大學獸醫學院動物疾病診斷中心進行組織切片，樣品經脫水、包埋、封蠟、切片後以蘇木精 - 伊紅染色 (hematoxylin and eosin staining) 並進行組織鏡檢，分析睪丸組織性狀。

VII. 統計分析

所有試驗數據以 mean ± S.E.M. 表示，並依據 SAS Dunnett's test 進行數據統計與差異顯著性檢測分析，當 $P < 0.05$ 表示有差異顯著性。

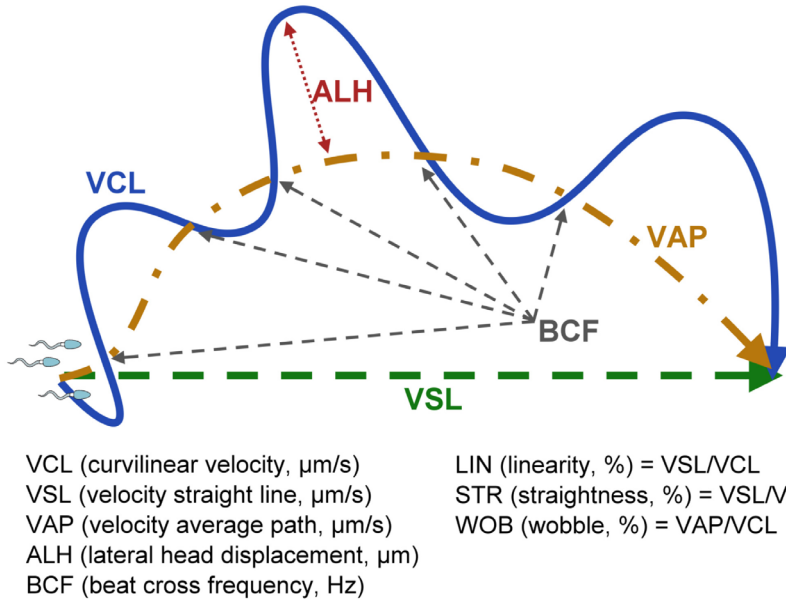

圖 1.　蘭嶼豬公豬精子活動能力分析。以 CASA 系統分析蘭嶼豬公豬精子的曲線移動速率 (curvilinear velocity, VCL; μm/s)、直線移動速率 (velocity straight line, VSL; μm/s)、平均移動路徑 (velocity average path, VAP; μm/s)、直線前進之比率 (linearity, LIN; VSL/VCL; %)、直線趨勢 (straightness, STR; VSL/VAP; %)、擺動性 (wobble, WOB; VAP/VCL; %)、精子平均側擺幅值 (lateral head displacement, ALH; μm)，以及精子頭部擺動與平均路徑交叉之頻率 (beat cross frequency, BCF; Hz) 等運動參數。

Fig. 1. Kinematic analysis of sperm in Lanyu pig boars. The VCL, VSL, VAP, LIN, STR, WOB, ALH, and BCF of sperm in Lanyu pig boars were analyzed by using CASA system.

結果與討論

本試驗將蘭嶼豬公豬飼養於常溫與高溫環境，並以 CASA 系統定期監測其精子性能變化，以瞭解高溫環境對其產精性狀表現之影響。試驗過程中，常溫組之豬欄溫度維持於 25 至 30℃，平均約 27℃；相對濕度維持於 60 至 95%，平均約 78%。高溫組之豬欄溫度維持於 30 至 35℃，平均約 32℃；相對濕度維持於 45 至 70%，平均約 59% (圖 2)。兩組豬欄溫度平均相差 5℃。藉由溫濕度數據，可換算為 THI 評估熱緊迫狀態。依據 Haeussermann *et al.* (2007) 研究指出，豬舍環境之 THI 於 74 以下為適宜狀態，75 至 78 為輕度熱緊迫，79 至 83 為中度熱緊迫，大於 84 為高度熱緊迫。本試驗常溫組 THI 介於 76 至 85，平均約 81，屬於中度熱緊迫程度；高溫組 THI 介於 85 至 94，平均約 90，屬於高度熱緊迫程度。

豬隻每 2 週自附睪採集精液，以 CASA 系統進行精子性狀分析之結果顯示，經 30 至 35℃之高溫環境處理 6 週後，蘭嶼豬公豬精子存活率已降至 69.5 ± 6.2%，8 週後存活率降至 62.6 ± 7.6%，前進式活力降至 54.3 ± 7.0%，而精子活力則降至 67.3 ± 6.8%，皆顯著低於常溫組 ($P < 0.05$) (表 1)。試驗第 8 週與第 0 週相比，高溫組公豬之精子存活率、前進式活力與精子活力，分別平均下降 25.1、24.1 及 23.6%。然而，高溫環境並未顯著影響精子 VCL、VSL、VAP、LIN、STR 與 WOB 等表現 (表 1)。此結果與前人研究結果相似，高溫環境會使精子品質變差 (Suriyasomboon *et al.*, 2005)。然而，不同品種的公豬，其精子性狀對於高溫環境的反應不盡相同，以商用豬種杜洛克、藍瑞斯與約克夏為例，藍瑞斯精子對於高溫環境較杜洛克與約克夏精子敏感，易於夏季高溫時發生異常 (郭等，1997；Kamanova *et al.*, 2021)。而源自中國廣西的巴馬香豬 (Bama miniature pigs)，則具有極高的耐熱性，夏季高溫並未顯著影響其精液品質 (Zhang *et al.*, 2015)。

蘭嶼豬經 30 至 35℃之高溫環境處理 8 週後，由上述結果可知高溫環境對精子造成不良影響，為了解高溫環境處理是否亦影響睪丸性狀，遂在試驗結束後採集睪丸進行組織切片分析。公豬的陰囊有散熱機制，使其睪丸溫度一般可低於體溫 2 至 8℃，以維持正常生精作用 (Shen *et al.*, 2019)；然而長期處於高溫環境，仍會逐漸危害睪丸性狀，最終影響精子性能。Parrish *et al.* (2017) 曾嘗試將睪丸進行熱處理，觀察到睪丸的生精小管 (seminiferous tubule) 產生大量空泡 (vacuoles)。本試驗結果顯示，試驗 8 週後常溫組睪丸長度約 7.8 ± 0.3 cm，高溫組睪丸長度則約 7.0 ± 0.2 cm，高溫組睪丸之尺寸大小顯著萎縮約 10% ($P < 0.05$) (圖 3)。將睪丸以縱切與橫切方式粗切，再進行石蠟包埋進

行組織切片染色鏡檢，可觀察到高溫組睪丸的生精小管已多處萎縮並空泡化 (圖 3)，顯示高溫環境的處理已破壞其造精能力，亦使精子品質降低。此結果與先前研究相似，高溫環境會減少精細胞的數量，並造成睪丸空泡化 (Pinart *et al.*, 2002)。

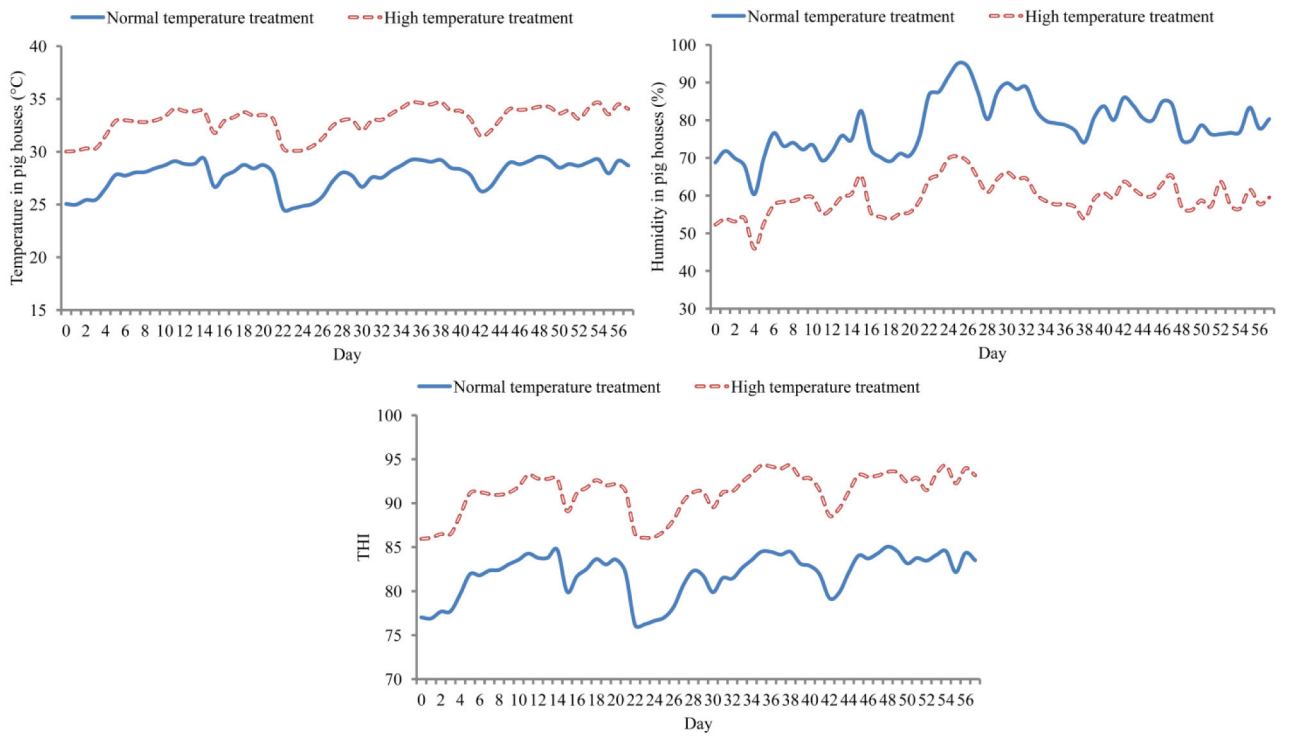

圖 2. 常溫與高溫組之溫濕度紀錄。豬欄以溫濕度計記錄 8 週的溫濕度變化。THI = [(1.8 × T) + 32] – [0.55 × (RH / 100)] × [(1.8 × T) + 32 – 58] (NWSCR, 1976)。

Fig. 2. Record of temperature and humidity in normal and high temperature groups. Thermohydrometers were used to monitor daily temperature and humidity changes in the pig houses for 8 weeks. THI = [(1.8 × T) + 32] – [0.55 × (RH / 100)] × [(1.8 × T) + 32 – 58] (NWSCR, 1976).

表 1. 蘭嶼豬精子性狀 #

Table1. Characteristics of Lanyu pig sperm#

	Normal temperature treatment (N = 4)				
Week	0	2	4	6	8
Viability (%)	80.3 ± 9.4	79.5 ± 5.90	75.6 ± 8.30	86.3 ± 1.8	83.4 ± 1.80
PR (%)	61.0 ± 0.1	78.0 ± 1.50	76.7 ± 3.00	74.5 ± 2.0	84.3 ± 0.90
Motility (%)	82.2 ± 5.3	87.3 ± 1.10	88.9 ± 1.90	88.3 ± 1.3	89.2 ± 1.80
VCL (μm/s)	80.1 ± 6.3	86.3 ± 13.9	77.0 ± 11.8	69.6 ± 8.2	87.4 ± 11.2
VSL (μm/s)	20.2 ± 2.3	22.2 ± 0.50	21.2 ± 0.90	19.1 ± 1.1	20.1 ± 0.70
VAP (μm/s)	43.1 ± 5.6	53.4 ± 7.50	45.7 ± 5.70	41.6 ± 4.6	51.9 ± 6.10
LIN (%)	36.1 ± 4.7	32.5 ± 6.60	31.8 ± 5.20	32.9 ± 1.9	24.0 ± 2.10
STR (%)	52.4 ± 4.9	48.0 ± 7.20	48.7 ± 4.90	54.1 ± 3.0	44.3 ± 4.90
WOB (%)	64.7 ± 2.3	64.8 ± 3.40	60.9 ± 3.30	58.5 ± 3.6	54.3 ± 0.80
ALH (μm)	3.0 ± 0.3	3.5 ± 0.50	3.4 ± 0.50	2.6 ± 0.2	3.9 ± 0.50
BCF (Hz)	7.4 ± 0.4	7.5 ± 0.50	6.3 ± 0.30	7.5 ± 0.4	9.6 ± 0.80
	High temperature treatment (N = 5)				
Week	0	2	4	6	8
Viability (%)	83.6 ± 11.2	76.6 ± 8.9	74.4 ± 10.8	69.5 ± 6.2*	62.6 ± 7.6*
PR (%)	71.5 ± 2.4	69.5 ± 3.1	69.9 ± 10.3	65.6 ± 4.2	54.3 ± 7.0*
Motility (%)	88.1 ± 3.6	84.1 ± 2.8	81.6 ± 4.3	75.5 ± 8.9	67.3 ± 6.8*
VCL (μm/s)	80.9 ± 6.0	82.5 ± 7.8	66.1 ± 13.3	68.2 ± 9.6	82.2 ± 12.1
VSL (μm/s)	23.5 ± 3.0	24.4 ± 1.9	23.3 ± 4.4	20.5 ± 2.9	17.6 ± 0.9
VAP (μm/s)	53.7 ± 7.6	52.0 ± 4.2	44.1 ± 9.8	44.9 ± 8.6	46.3 ± 6.0

表 1.　蘭嶼豬精子性狀 #(續)

Table1.　Characteristics of Lanyu pig sperm# (continued)

	High temperature treatment (N = 5)				
Week	0	2	4	6	8
LIN (%)	30.9 ± 3.2	34.3 ± 3.4	38.5 ± 1.3	34.5 ± 2.4	25.0 ± 2.4
STR (%)	47.8 ± 4.9	50.7 ± 4.7	54.9 ± 1.9	50.1 ± 2.7	44.1 ± 5.8
WOB (%)	61.2 ± 0.7	64.9 ± 1.6	66.7 ± 1.6	65.4 ± 5.0	57.1 ± 5.7
ALH (μm)	3.7 ± 0.4	3.4 ± 0.3	2.8 ± 0.5	2.7 ± 0.3	3.2 ± 0.3
BCF (Hz)	7.1 ± 0.2	6.8 ± 0.2	5.6 ± 0.6	6.6 ± 0.6	8.2 ± 1.1

The semen of Lanyu pig boars was collected from epididymis every 2 weeks, and sperm characteristics were analyzed by using CASA system.

PR, progressive motility; VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average path velocity; LIN, linearity; STR, straightness; WOB, wobble; ALH, amplitude lateral head; BCF, beat frequency.

* P < 0.05 versus Normal temperature treatment at the same week.

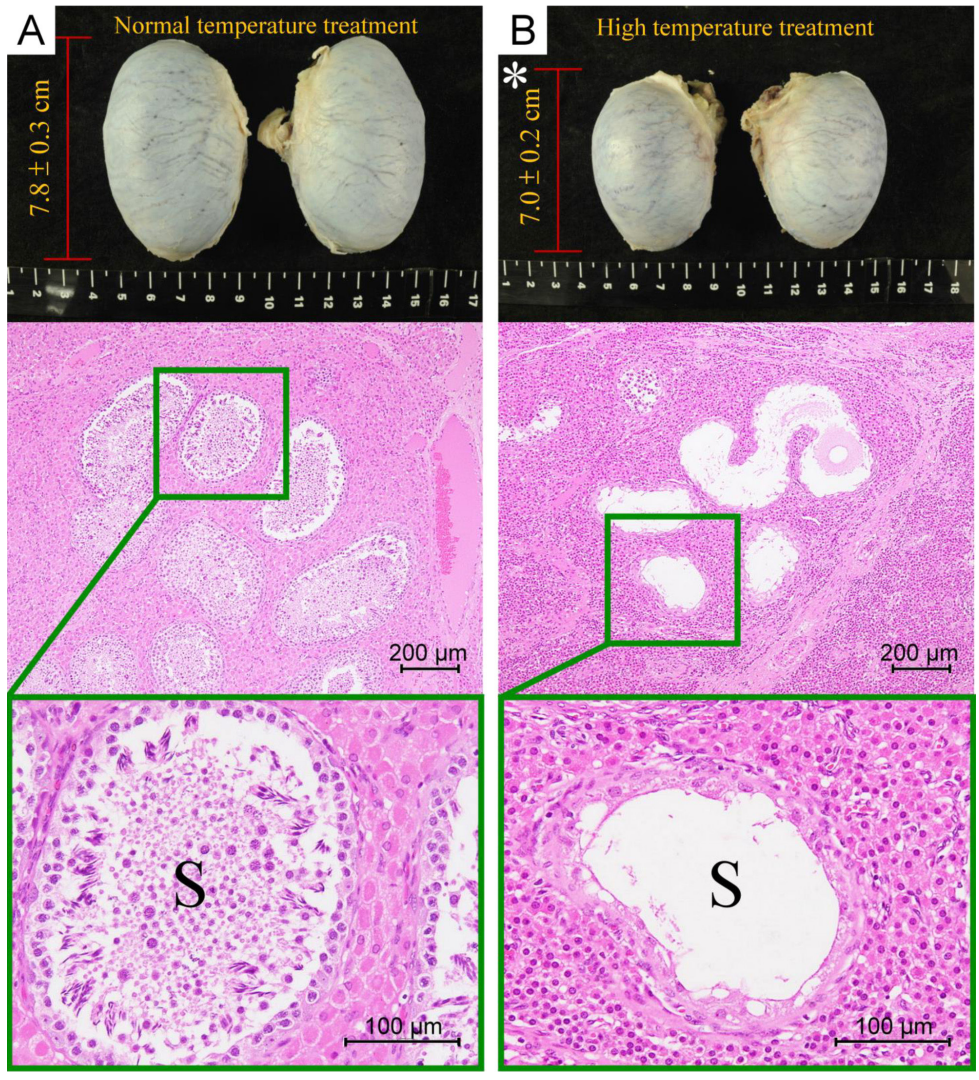

圖 3.　蘭嶼豬睪丸長度與生精小管性狀。蘭嶼豬公豬睪丸於試驗第 8 週採集，進行組織切片與蘇木精－伊紅染色檢測睪丸性狀。(A) 常溫組睪丸長度約 7.8 ± 0.3 cm，且睪丸生精小管結構完整；(B) 高溫組睪丸長度約 7.0 ± 0.2 cm，顯著縮小約 10%，且生精小管萎縮及空泡化。S，生精小管。* P < 0.05 與常溫組有顯著差異。

Fig. 3.　Length of testes and characteristics of seminiferous tubule in Lanyu pigs. The testes of Lanyu pig boars were collected at the 8th week of the experiment, and their characteristics were examined by tissue section and hematoxylin and eosin staining. (A) The testis in the normal temperature group was 7.8 ± 0.3 cm in length, and the structure of seminiferous tubule was intact; (B) The testis in the high temperature group was 7.0 ± 0.2 cm in length, showing about 10% atrophy, and the structure of their seminiferous tubule was found atrophy and vacuolation. S, seminiferous tubule. * P < 0.05 versus Normal temperature treatment.

綜觀以上之結果，蘭嶼豬公豬長期處於 30 至 35℃之高溫環境，精子活力減少 23.6%，睪丸萎縮 10%。源起於臺灣離島蘭嶼之蘭嶼豬，耐熱性較一般洋豬佳 (陳，2020)。自然環境下，日夜氣溫與環境溫度有高低變化，蘭嶼豬若處於白天炎熱夜間涼爽的常溫環境下，仍可保有一定的繁殖力。然本試驗結果證實，若日夜皆處高溫，延長了豬隻處於熱緊迫時間，長期下來會嚴重危害蘭嶼豬公豬之產精性能。全球暖化使得處於高溫高濕環境的機率越來越高，對於散熱條件欠佳的豬舍是一大挑戰。而蘭嶼豬公豬飼養時，避免長期處於 30℃以上之高溫環境，則可避免繁殖性能的降低。

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Reproductive performance of Lanyu pig boars under high environmental temperature (1)

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Abstract

The reproductive traits of boars depend on internal factors such as age, breed, and nutrition, as well as external factors such as animal care and housing conditions. Semen quality is closely related to environmental temperature although different types of boar breed exhibit different sensitivity to temperature changes. To understand the effect of high environmental temperature on the reproductive traits of Lanyu pig boars, the present study was conducted in the pig barn with normal temperature (30-35°C) – 81 of average temperature humidity index (THI) and with high temperature (30-35°C) – 90 of average THI for 8 weeks. The semen was collected from epididymis every 2 weeks, and sperm characteristics were analyzed by using computer-assisted sperm analysis (CASA) system. The results show that in the high temperature group (high heat stress environment), sperm survival rate dropped to 69.5 ± 6.2% after 6 weeks of treatment; and after 8 weeks of treatment, the sperm survival rate, progressive motility (PR), and sperm motility were decreased to 62.6 ± 7.6%, 54.3 ± 7.0%, and 67.3 ± 6.8%, respectively. All of above parameters in high temperature group were significantly lower than those in the normal temperature group (moderate heat stress environment) (83.4 ± 1.8%, 84.3 ± 0.9%, and 89.2 ± 1.8%, respectively). The sperm survival rate, PR, and sperm motility in the high temperature group decreased by an average of 25.1%, 24.1%, and 23.6%, respectively, when compared with the results between week 8 and week 0. After 8 weeks of high temperature treatment, the testicular size was 7.0 ± 0.2 cm, which showed a significant shrinkage of about 10% compared with 7.8 ± 0.3 cm in the normal temperature group. The tissue section results showed that the seminiferous tubules also exhibited atrophy and vacuolation, indicating that the high environmental temperature has destroyed their sperm production ability. Therefore, long-term exposure to high environmental temperature will decrease sperm mortality rate, reduce sperm motility, and cause testicular atrophy in Lanyu pig boars. The results of this study reveal thermal deterioration effect of high environmental temperature on the reproductive trait in Lanyu pig boars.

Key words: Lanyu pigs, High environmental temperature, Reproductive traits, Sperm, Testis.

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