豬冷凍精液解凍溫度及時間之探討(1)

章嘉潔(2)(3)

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摘要

本試驗探討四種解凍條件（50℃, 50 秒、45℃, 50 秒、50℃, 45 秒及 45℃, 45 秒） 對豬冷凍精液解凍後品質的影響。選擇生殖能力正常、平均年齡 2.5 歲之健壯杜洛克公豬 8 頭，採集其精液製作冷凍精液，並比較解凍條件對解凍後之精子存活率、精子總活力、精子快速前進式活力等項目之差異。試驗結果顯示，解凍條件分別為 50℃, 50 秒及 50℃, 45 秒，對於解凍後精子之存活率、精子之總活力、精子之快速前進式活力等，在統計上均無顯著差異；而解凍條件為50℃, 45 秒，則對於解凍後之精子存活率、精子總活力、精子快速前進式活力等均顯著優於解凍條件為 45℃, 45 秒組（$P < 0.05$）。

關鍵詞：精液、解凍、豬。

緒言

精液冷凍保存之目的為充分發揮優良種公畜的遺傳潛能，延長精子的貯存時間及維持其受精能力，以便於長途運輸引種，降低疫病的傳播風險，加快畜群品種改良及育種工作，同時也是建立種源基因庫不可缺少的技術，特別是對於瀕危物種資源，精液冷凍保存更是絕對必備之技術（Glossop, 1997; Bailey *et al.,* 2008）。

豬之精子對低溫特別敏感（Tamuli and Waston,1994; Hofmo and Grevle, 2000），因此在冷凍解凍過程中極易受物理及化學性的損害，而降低精子之存活及受孕率（Hammerstedt *et al.,* 1990; Waberski *et al.,* 1994），以致豬冷凍精液在養豬產業中一直難以大範圍推廣應用。豬冷凍精液需要進一步改善者，包括冷凍之溫度、時間（Gadea *et al.,* 2005; Holt *et al.,* 2005; Hernández *et al.,* 2007）、稀釋液（Fraser and Strzezek *et al.,* 2007; Grossfeld *et al.,* 2008）、處理流程與裝填（Buranaamnuay *et al.,* 2008; Ekwall *et al.,* 2009）等 。另外，在解凍方面對精子存活率有不同程度影響（Cordova-Izquierdo *et al.,* 2006），而亦有研究發現冷凍速率不會影響精液之品質，但解凍速率則會影響精液之品質（Hernández *et al.,* 2007）。Fiser *et al.*（1993）以裝填於 0.5 mL 麥管方式

（1）行政院農業委員會畜產試驗所研究報告第 1516 號。
（2）行政院農業委員會畜產試驗所台東種畜繁殖場。
（3）通訊作者，E-mail: janices@mail.tlri.gov.tw。

進行測試，結果建議解凍回溫速率為 1200℃/min。但實際商業應用，現場操作時均以 5 ml 大麥管方式冷凍，研究將解凍溫度增加至 50℃ 或 60℃ 時較 37℃ 能使精子活力有更佳的表現（Salamon *et al.,*1973）。5 ml 麥管以 52℃, 40 秒至 52 秒解凍均有研究報告，如以 52℃, 40 秒解凍後，麥管內溫度約為 18℃; 50℃, 45 秒解凍後為 24℃; 52℃, 52 秒解凍後，麥管溫度將約為 32-34℃（Aumuller, 1982; Pursel and Park,1985）。由於目前研究大多數集中在探討冷凍技術上，而對解凍技術的研究相對較少，因此依現有之知識仍然無法實際應用。

精子活力常用來做為精子品質之指標，同時也是決定授精的重要因素，傳統測定家畜精子存活率是使用目測方法，現場應用簡單方便。但該方法主觀性強，與受胎率相關性差（Saacke and White, 1972; Kommisrud *et al.,* 2002）。近年來，哺乳動物的精液質量分析上廣泛應用精液分析儀（Mortimer *et al.,* 1997; Verstegen *et al.,* 2002）， 此儀器可提供客觀、準確模式，評估精子活力、精子直線轉動速率、平均路線速度等特性的測定，是一種在無損害代謝與膜完整性下的安全指標（Mortimer, 2000; Amann and Katz, 2004）。一些研究顯示受胎率與精子活動速率呈現相關性，受胎率可藉不同形式速率參數進行預測（Liu and Baker, 1992; Barrat *et al.,* 1993; Holt *et al.,* 1997）。

本試驗選擇目前已廣泛使用於田間之精液稀釋液，探討不同解凍溫度與時間，對解凍後精子存活率、總活力、快速前進式活力之影響，篩選出保存效果較佳之解凍條件，以延長精子之存活時間，期為豬精液冷凍保存及生產之推廣應用提供參考。

材料與方法

Ⅰ. 精液採集與處理

本試驗選育生殖能力正常、平均年齡 2.5 歲之健壯的杜洛克公豬 8 頭。每週採集精液一次，精液採集後立即進行常規檢查，並在 37℃ 下靜置 30 分鐘後，再選取存活率 80% 以上與活力 75% 以上之精液進行冷凍保存。

Ⅱ. 精液之冷凍

採集之精液選取濃厚部份，並鏡檢精液品質，再使用 Beltsville thawing solution（BTS, 37.0 g/L glucose, 1.25 g/L EDTA, 6.0 g/L sodium citrate, 1.25 g/L sodium bicarbonate, and 0.75 g/L potassium chloride）（Johnson *et al.,* 2000）進行精液稀釋。稀釋之精液先冷卻至 15℃，維持 3 小時 ，然後於 15℃ 下 800×g, 離心 10 分鐘 ，經去除上清液後添加冷凍稀釋液（Ⅰ）（11% lactose 及 20% egg yolk）稀釋，然後再冷卻至 5℃，維持 2 小時；再使用冷凍稀釋液（Ⅱ）（11% lactose, 20% egg yolk, 9% glycerol 及 1.5% Equex STM）稀釋，使最終精子濃度為 1×10^9 cells/mL，經裝填於 5 mL 之麥管，並以塑膠珠封住兩端。其次將麥管移置於電腦程式控制儀（Ice cube 14S，Minitub）內，以冷凍程式降溫，其中 5℃ 至 -5℃，以每分鐘下降 3℃ 之速率進行，最後將完成冷凍之麥管移入液氮桶內貯存（Westendorf *et al.,* 1975；Bwanga *et al.,* 1991）。

Ⅲ. 冷凍精液之解凍過程

備妥解凍用稀釋液，使回溫至 25℃，並迅速從液氮桶內取出所需之冷凍精液，將麥管依不同解凍時間及溫度條件進行解凍。擦乾麥管表面之水分，先剪開麥管之一端，其次再剪開另一端，讓精液流入 80 ml（25℃）之解凍用稀釋液中，然後進行解凍後之精液性狀測試，並於 37℃ 靜置 7 小時。靜置期間儘可能避免精液受到溫度變化及光線傷害，並使用顯微鏡及精液分析儀評估精液之

品質，項目包括：精子存活率、精子總活力、精子快速前進式活力。

Ⅳ. 精液性狀之評估

精液解凍後，精液品質之檢測使用 CASA 系統（VideoTesT-Sperm 2.1, VideoTesT-Metel, Russia），其方法為取 5 μL 含解凍稀釋液之精液置於 Makler counting chamber（Makler, OC, Microcell etc.）進行評估。精子活力指數依 WHO（World Health Organization）之標準:（1）總活力定義為細胞移動速率 VAP > 10 μm/sec;（2）快速前進式活力（rapid progressive motility, RPM）定義為細胞移動速率 VAP > 25 μm/sec，受測溫度為 37℃。精子存活率之評估，取原精液製成抹片，以伊紅-尼格羅黑（eosin-nigrosin）染劑進行染色，經快速風乾後置於 400 倍顯微鏡下鏡檢，若染成紅色者即代表死精子。每一抹片計算 200 隻精子，並以活精子數除以總精子數，即為精子之存活率。

Ⅴ. 統計分析

豬冷凍精液使用不同稀釋液解凍後，靜置於 37℃, 7 小時，並評估其存活率、總活力及快速前進式活力；而試驗所得資料以一般線性模式（General Linear Model Procedure, GLM）及鄧肯式新多變域測定法（Duncan's New Multiple Range Test）比較解凍後精液各性狀間總平均值之差異顯著性（ SAS, 2005）。

結果與討論

研究公豬精液冷凍解凍關鍵技術，改進公豬精液冷凍解凍方法，為國內、外養豬生產中急待解決的問題。本試驗使用公豬冷凍精液比較不同解凍條件，對解凍後之精子存活率、精子活力 、精子快速前進式活力之影響。表 1 為不同精液樣品經靜置培養，解凍後之精子存活率、精子活力 、精子前進式活力呈現顯著差異（P < 0.0001）。先前報告證實公豬個體間及冷凍精液保存均呈現顯著差異（Roca *et al.,* 2006; Hernández *et al.,* 2007）；推論原因可能與公豬個別基因之差異，以致影響精子之特性及精漿所致（Thurston *et al.,* 2002; Okazaki *et al.,* 2009）。如表 1 之結果，解凍後之精液靜置培養 7 小時各時間之間距，其精液性狀呈現統計上之顯著差異（P < 0.0001）；精液性狀均隨儲存時間之延長而降低，此與 Dube *et al.*（2004）結果一致。不同解凍溫度、時間條件組，精液性狀均呈現統計顯著差異（P < 0.01），表示不同解凍條件是有所差異。靜置培養時間與不同解凍條件組間，解凍後之精子存活率、精子活力 、精子快速前進式活力並無顯著差異及交感作用。

表 1. 統計模式中精子品質參數之顯著值

Table 1. Levels of significance for the effects included in the statistical model based on sperm quality parameters

Source of variation	Degrees of freedom	Viability	Total motility	RPM*
Swine semen sample	7	<0.0001	<0.0001	<0.0001
Storage time	7	<0.0001	<0.0001	<0.0001
Method	3	0.0018	<0.0001	<0.0001
Storage time × method	21	0.9726	0.9796	0.6750

* RPM, Rapid progressively motility.

以解凍溫度、時間條件分別為 50℃, 50 秒、45℃, 50 秒、50℃, 45 秒及 45℃, 45 秒進行測試包括精子存活率、精子總活力、精子快速前進式活力，試驗結果如表 2、3 及 4。由結果顯示，解凍條件為 50℃, 50 秒及 50℃, 45 秒二者之精液性狀無顯著差異，而 50℃, 50 秒及 45℃, 50 秒二者之精液性狀亦無顯著差異；其中並以 50℃, 45 秒之精液性狀顯著優於 45℃, 45 秒（$P < 0.05$）。

表 2. 不同解凍條件對公豬冷凍精液解凍後精子存活率之影響

Table 2. Effects of different thawing conditions on sperm viability in frozen-thawed boar semen

Treatment	Post-thaw sperm viability(%)								Mean
	Storage time								
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	
50℃ 50s	53.78^{a}	50.08^{ab}	43.59^{a}	36.90^{a}	35.49^{a}	26.64^{a}	23.19^{a}	20.05^{a}	36.94^{ab}
50℃ 45s	59.45^{a}	53.04^{a}	45.08^{a}	38.38^{a}	34.41^{a}	31.86^{a}	25.08^{a}	25.30^{a}	39.07^{a}
45℃ 45s	56.25^{a}	43.04^{b}	41.22^{a}	33.80^{a}	29.48^{a}	26.28^{a}	21.99^{a}	19.48^{a}	33.94^{b}
45℃ 50s	59.65^{a}	47.14^{ab}	43.34^{a}	40.49^{a}	33.43^{a}	29.98^{a}	27.11^{a}	20.11^{a}	37.66^{ab}

^{a,b} Means with different superscripts are different significantly ($P < 0.05$).

表 3. 不同解凍條件對公豬冷凍精液解凍後精子總活力之影響

Table 3. Effects of different thawing conditions on sperm total motility in frozen-thawed boar semen

Treatment	Post-thaw sperm total motility (%)								Mean
	Storage time								
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	
50℃ 50s	45.15^{ab}	33.76^{b}	30.20^{a}	27.77^{a}	21.69^{a}	15.69^{a}	12.18^{ab}	9.61^{ab}	24.46^{ab}
50℃ 45s	46.57^{a}	40.25^{a}	33.65^{a}	25.88^{a}	22.11^{a}	18.49^{a}	15.09^{a}	12.25^{a}	26.78^{a}
45℃ 45s	37.95^{c}	28.17^{b}	23.34^{b}	19.82^{b}	15.57^{b}	12.01^{b}	8.98^{b}	5.92^{b}	18.98^{c}
45℃ 50s	45.57^{ab}	32.90^{b}	27.95^{ab}	24.18^{ab}	20.23^{ab}	16.25^{ab}	13.45^{ab}	7.71^{ab}	23.52^{b}

^{a,b} Means with different superscripts are different significantly ($P < 0.05$).

表 4. 不同解凍條件對公豬冷凍精液解凍後精子快速前進式活力之影響

Table 4. Effects of different thawing conditions on sperm rapid progressively motility in frozen-thawed boar semen

Treatment	Post-thaw sperm RPM* (%)								Mean
	Storage time								
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	
50℃ 50s	25.58^{a}	21.05^{a}	13.37^{a}	12.87^{a}	13.75^{a}	7.92^{a}	5.37^{a}	4.85^{a}	13.09^{a}
50℃ 45s	24.05^{a}	24.58^{a}	18.50^{a}	11.67^{a}	11.50^{ab}	8.70^{a}	7.23^{a}	7.87^{a}	14.26^{a}
45℃ 45s	21.75^{a}	11.77^{b}	12.90^{a}	7.25^{a}	5.72^{b}	5.73^{a}	2.63^{a}	2.35^{a}	8.76^{b}
45℃ 50s	26.50^{a}	14.17^{b}	13.53^{a}	13.40^{a}	8.93^{ab}	6.35^{a}	6.35^{a}	3.55^{a}	11.60^{ab}

^{a,b} Means with different superscripts are different significantly ($P < 0.05$).

* RPM, Rapid progressively motility.

豬冷凍精液目前廣泛使用 5 mL 麥管裝填（Almid and Hofmo, 1996），但因中心之熱傳導速率較慢，易形成一溫度梯度（Bwange *et al.,* 1990），而引起精子功能與生化損傷等問題。5 mL 麥管之解凍方式，解凍溫度範圍為 35-90℃，時間範圍為 6-120 秒（Pursel and Park, 1987）。家畜精子在冷凍解凍過程，會經 -15℃ 至 -60℃ 臨界溫度區，明顯造成傷害（Eriksson and Rodriguez-Martinnez, 2000）；此在綿羊（Fiser *et al.,* 1986）、牛（De Abreu *et al.,* 1979）、豬（Westendorf *et al.,* 1975; Fiser *et al.,* 1993）之研究已分別被證實。

解凍之速率是影響精子存活率之重要因素，而快速解凍有益提升精子之存活率及頭帽完整性，並改善活力（Eriksson and Rodriguez-Martinnez, 2000; Cordova-Izquierdo *et al.,* 2006）。Salamon *et al.*（1973）之研究，發現解凍溫度上升至 50℃ 或 60℃ 時，精液性狀較 37℃ 為佳。解凍溫度為 52℃，而水浴時間分別為 28、34、40、46、52 及 58 秒，結果以 40 秒組之解凍精液有較佳的活力與正常頭帽比率（Pursel and Park, 1985）。Selles *et al.*（2003）將豬冷凍精液之解凍速率提升，則可顯著提高體外培養之受胎率。Cordova *et al.*（2006）發現以 50℃, 40 秒解凍之精液，其精子活力優於 42℃, 40 秒解凍者（$P < 0.05$）。Hernández *et al.*（2007）發現降溫速率不會影響精子品質之參數，而甘油濃度與回溫速率卻會顯著影響解凍精子之品質參數。目前國內商業用進口公豬冷凍精液，解凍條件有 47℃, 45 秒、50℃, 50 秒、50℃, 45 秒等，而本研究使用國內自行製作冷凍精液，以 50℃, 45 秒解凍，在精子存活率、精子活力、精子快速前進式活力等均優於以 45℃, 45 秒解凍者（$P < 0.05$），因此提升回溫速率確能顯著改善精液之性狀。

影響解凍後精子活力及存活率之機制（Mazur *et al.,* 1972; Pursel *et al.,* 1972），不外與冰晶再生及細胞死亡有關（Mazur, 1985），因此如欲減少損失必須改善解凍之步驟與過程，提升解凍速率，有效減少再結晶及冰晶之形成（Courtens and Paquignon, 1985; Fiser *et al.,* 1993）；但回溫速率太快，也會影響滲透壓之平衡（Mazur, 1984），造成細胞內外液體、冷凍保護劑之流動，而導致細胞腫脹或水解（Mazur, 1972; Bwanga *et al.,* 1991）。解凍速率之最佳方式，必須避免細胞內冰晶再形成，使再結晶的小冰晶數目減至最少，並使細胞處在高濃度溶質中的時間縮至最短，以減少細胞的物理及滲透壓傷害，方能維護其正常生理功能（Mazur, 1977; Watson, 1979）。

由本研究之結果，顯示以 50℃, 45 秒解凍，對精子存活率、精子活力、精子快速前進式活力等之精液性狀，確實具有顯著改善之效果，因此本解凍條件可作為產業推廣公豬冷凍精液應用之參考。

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參考文獻

Amann, R. P. and D. F. Katz. 2004. Reflections on CASA after 25 years. J. Androl. 25: 317-325.

Almlid, T. and P. O. Hofmo. 1996. A brief review of frozen semen application under Norwegian AI service conditions. Reprod. Dom. Anim. 31: 169-173.

Aumuller, R. 1982. Tiefgefrierung von Ebersamen in Kunststoffrohren. Uberprufung des Einflusses der

Zentrifugationstechnik im Besamungsversuch unter Praxisbedinungen.Thesis, Tierarztliche Hochschule, Hannover, Germany.

Bailey, J. L., C. Lessard, J. Jacques, C. Bréque, I. Dobrinski, W. Zeng and H. L. Galantino-Homer. 2008. Cryopreservation of boar semen and its future importance to the industry. Theriogenology 70: 1251-1259.

Barrat, C. L. R., M. J. Tomlinson and I. D. Cooke. 1993. Prognostic significance of computerized motility analysis for in vivo fertility. Fertil. Steril. 60: 520-525.

Buranaamnuay, K., P. Tummaruk, J. Singlor, H. Rodriguez-Martinez and M. Techakumphu. 2009. Effects of straw volume and Equex-STM on boar sperm quality after cryopreservation. Reprod. Domest. Anim. 44: 69-73.

Bwanga, C. O., S. Einarsson and H. Rodriguez-Martinez. 1991. Cryopreservation of boar semen. II: Effect of cooling rate and duration of freezing point plateau on boar semen frozen in mini- and maxi-straws and plastic bags. Acta. Vet. Scand. 32: 455-461.

Bwanga, C. O., M. M. deBraganca, S. Einarsson and H. Rodriguez-Martinez. 1990. Cryopreservation of boar semen in Mini-and Maxi-straws. J. Vet. Med. 37: 651-658.

Cordova-Izquierdo, A., J. H. Oliva, B. Lleó, C. García-Artiga, B. D. Corcuera and J. F. Pérez-Gutiérrez. 2006. Effect of different thawing temperatures on the viability, in vitro fertilizing capacity and chromatin condensation of frozen boar semen packaged in 5 ml straws. Anim. Reprod. Sci. 92: 145-154.

Courtens, J. L. and M. Paquignon. 1985. Ultrastructure of fresh, frozen and frozen-thawed spermatozoa of the boar. In: Deep freezing of boar semen. Swedish University of Agricultural Science, Uppsala, pp. 61-87.

DeAbreu, R. M., W. E. Berndston, R. L. Smith and B. W. Pickett. 1979. Effect of post-thaw warming on viability of bovine spermatozoa thawed at different rates in fresh straws. J. Dairy Sci. 62: 1449-1454.

Dubé, C., M. Beaulieu, C. Reyes-Moreno, C. Guillemette and J. L. Bailey. 2004. Boar sperm storage capacity of BTS and Androhep Plus: Viability, motility capacitation, and tyrosine phosphorylation. Theriogenology 62: 874-886.

Ekwall, H. 2009. Cryo-scanning electron microscopy discloses differences in dehydration of frozen boar semen stored in large containers. Reprod. Domest. Anim. 44: 62-68.

Ekwall, H., M. Hernandez, F. Saravia and H. Roderiguez-Martinez. 2007. Cryo-scanning electron microscopy (Cryo-SEM) of boar semen frozen in medium-straws and mini flat packs. Theriogenology 67: 1463-1472.

Eriksson, B. M. and H. Rodriguez-Martinez. 2000. Effect of freezing and thawing rates on the post-thaw viability of boar spermatozoa frozen in Flat Packs and Maxi-straws. Anim. Reprod. Sci. 63: 205-220.

Fiser, P. S., R. W. Fairful and G. J. Marcus. 1986. The effect of thawing velocity on survival and acrosomal integrity of ram spermatozoa frozen at optimal and suboptimal rates in straws. Cryobiology 23: 141-149.

Fiser, P. S., R. W. Fairful, C. Hansen, P. L. Panich, J. N. B. Shrestha and L. Underhill. 1993. The effect of warming velocity on motility and acrosome integrity of boar sperm as influenced by the rate of freezing and glycerol level. Mol. Reprod. Dev. 34: 190-195.

Fraser, L. and J. Strzezek. 2007. Effect of different procedures of ejaculate collection, extenders and packages on DNA integrity of boar spermatozoa following freezing-thawing. Anim. Reprod. Sci. 99: 317-329.

Gadea, J., F. García-Vazquez, C. Matás, J. C. Gardón, S. Cánovas and D. Gumbao. 2005. Cooling and freezing of boar spermatozoa: Supplementation of the freezing media with reduced glutathione preserves sperm function. J. Androl. 26: 396-404.

Glossop, C. E. 1997. The use of fresh and frozen semen in the swine industry. In: Proceedings of the Annual Meeting on Society of Theriogenology, Montreal, Canada. pp. 193-197.

Grossfeld, R., B. Sieg, C. Struckmann, A. Frenzel, W. M. Maxwell and D. Rath. 2008. New aspects of boar semen freezing strategies. Theriogenology 70: 1225-1233.

Hammerstedt, R. H., J. K. Graham and J. P. Nolan.1990. Cryopreservation of mammalian sperm: What we ask them to survive. J. Androl. 11: 73-88.

Hernández, M., J. Roca, M. A. Gil, J. M. Vázquez and E. A. Martínez. 2007. Adjustments on the cryopreservation conditions reduce the incidence of boar ejaculates with poor sperm freezability. Theriogenology 67: 1436-1445.

Hofmo, P. O. and I. S. Grevle. 2000. Development and commercial use of frozen boar semen in Norway. Boar Semen Preservation IV, pp. 71-77.

Holt, C., W. V. Holt, H. D. M. Moor, H. C. B. Reed and R. M. Curnock. 1997. Objectively measured boar sperm motility parameters correlate with the outcomes of on-farm inseminations: results of two fertility trials. J. Androl. 18: 312-323.

Holt, W. V., A. Medrano, L. M. Thurston and P. F. Watson. 2005. The significance of cooling rates and animal variability for boar sperm cryopreservation: Insights from the cryomicroscope. Theriogenology 63: 370-382.

Kommisrud, E., H. Paulenz, E. Sehested and I. S. Grevle. 2002. Influence of boar and semen parameters on motility and acrosome integrity in liquid boar semen stored for five days. Acta. Vet. Scand. 43: 49-55.

Liu, D. Y. and H. W. G. Baker. 1992. Tests of human sperm function and fertilization in vitro. Fertil. Steril. 58 : 465-483.

Mazur, P., S. P. Leibo and E. H. Y. Chu. 1972. A two factor hypothesis of freezing injury. Exp. Cell Res. 71: 345-355.

Mazur, P. 1977. The role of intracellular freezing in the death of cells cooled at supraoptimal rates. Cryobiology 14: 251-272.

Mazur, P. 1984. Freezing of live cells: Mechanisms and implications. Am. J. Physiol. 247: 125-142.

Mazur, P. 1985. Basic concepts in freezing cells. In: Deep Freezing of Boar Semen. Johnson, L. A, Lasson and K.（Eds.）Swedish University of Agricultural Sciences, Uppsala, Sweden, pp. 91-111.

Mortimer, S. T. 1997. A critical review of the physiological importance and analysis of sperm movement in mammals. Hum. Reprod. Update 3: 403-439.

Mortimer, S. T. 2000. CASA-practical aspects. J. Androl. 21: 515-524.

Okazaki, T., S. Abe, S. Yoshida and M. Shimada. 2009. Seminal plasma damages sperm during cryopreservation, but its presence during thawing improves semen quality and conception rates in boars with poor post-thaw semen quality. Theriogenology 71: 491-498

Pursel, V. G., L. A. Johnson and G. B. Rampacek. 1972. Acrosome morphology of boar spermatozoa incubated before cold shock. J. Anim. Sci. 34: 278-283.

Pursel, V. G. and C. S. Park. 1985. Freezing and thawing procedures for boar spermatozoa. In: Deep Freezing of Boar Semen. Johnson, L.A., Larsson, K. eds : Swedish University of Agricultural Science, Uppsala, Sweden. pp. 147-166.

Pursel, V. G. and C. S. Park. 1987. Duration of thawing on post-thaw acrosome morphology and motility of boar spermatozoa frozen in 5-ml maxi-straws. Theriogenology 28: 683-690.

Roca, J., M. Hernández, G. Carvajal, J. M. Vázquez and E. A. Martínez. 2006. Factors influencing boar sperm

cryosurvival. J. Anim. Sci. 84: 2692–2699.

Saacke, R. G. and J. M. White. 1972. Semen quality tests and their relationship to fertility. In: Proceedings of 4th Technical Conference on Artificial Insemination and Reproduction, National Association of Animal Breeders, Chicago, pp. 22-27.

Salamon, S., I, Wilmut and C. Polge. 1973. Deep freezing of boar semen. I. Effects of diluent composition, protective agents, and method of thawing on survival of spermatozoa. Aust. J. Biol. Sci. 26: 219-230.

SAS. 2005. User's Guide: Statistics, Version 9.1 Edition. SAS Inst. Inc., Cary, NC.

Selles, E., R. Gadea, C. Matas and S. Ruiz. 2003. Analysis of in vitro fertilizing capacity to evaluate the freezing procedures of boar semen and to predict the subsequent fertility. Reprod. Dom. Anim. 38: 66-72.

Tamulim, M. K. and P. F. Waton. 1994. Cold resistance of live boar spermatozoa during incubation after ejaculation. Vet. Rec. 135: 160-162.

Thurson, L. M., K. Siggins, A. J. Milleham, P. F. Watson and W. V. Holt. 2002. Identification of amplified restriction fragment length polymorphism markers linked to genes controlling boar sperm viability following cryopreservation. Biol. Reprod. 66: 545-554.

Verstegen, J., M. Jguer-Ouada and K. Onclin. 2002. Computer assisted semen analyzers in andrology research and veterinary practice. Theriogenology 57: 149-179.

Waberski, D., S. Meding, G. Dirksen, K. F. Weitze, C. Leiding and R. Hahn. 1994. Fertility of long-term-stored boar semen: Influence of extender (Androhep and Kiev), storage time and plasma droplets in the semen. Anim. Reprod. Sci. 36: 145-151.

Watson, P. F. 1979. The preservation of semen in mammals. In: Oxford review of reproductive biology, Finn, C.A. ed, Vol. 1, pp. 238-350, Oxford University Press, Oxford.

Westendorf, P., L. Richter and H. Treu. 1975. Zur Tiefgefrierung von Ebersperma Labor-und Besamungsergebnisse mit dem Hulsenberger Pailetten-Verfahren. Dtsch. Tierarztl. Wschr. 82: 261-267.

Woelders, H., A. Matthijs, C. A. Zuidberg and A. E. Chaveiro. 2005. Cryopreservation of boar semen: Equilibrium freezing in the cryomicroscope and in straws. Theriogenology 63: 383-395.

Effects of thawing temperature and time on boar frozen semen(1)

Chia-Chieh Chang(2)(3)

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Abstract

This experiment was conducted to investigate the effects of thawing conditions at 50℃, 50 sec, 45℃, 50 sec、50℃, 45 sec and 45℃, 45 sec respectively on quality of frozen- thawed semen in boars. Semen collected from eight healthy Duroc boars of known reproductive history (2.5 year of age), were retained for sperm cryopreservation. Frozen semen with different thawing conditions and the percentage of live sperm, total motility and rapid progressive motility (RPM) were investigated. The results showed that the percentages of live sperm, total motility and RPM were not significantly different between the two groups of thawed at 50℃, 50 sec and 50℃, 45 sec. ($P > 0.05$). Thawing straw at 50℃, 45 sec had significantly ($P < 0.05$) higher percentage of live sperm, total motility and RPM than those of straws thawed at 45℃, 45 sec.

Key words : Semen, Thawing, Boar.

（1）Contribution No.1516 from Livestock Research Institute, Council of Agriculture, Executive Yuan.
（2）Taitung Animal Propagation Station, COA-LRI, Taitung, 954, Taiwan, R.O.C.
（3）Corresponding author, E-mail: janices@mail.tlri.gov.tw