

山羊腹腔鏡人工授精之研究⁽¹⁾

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摘要

本研究主要在建立山羊腹腔鏡人工授精技術，評估懷孕率、總產仔數與每頭母羊之平均產仔數，並與傳統人工授精進行比較。試驗母羊經發情同期化處理，分別施以傳統人工授精及腹腔鏡人工授精，授精後第 45 天藉由超音波妊娠診斷儀器檢測是否懷孕。試驗結果使用傳統人工授精方式授精 30 頭母羊，懷孕率為 60% (18/30)，母羊之總產仔數為 42 頭 (26 ♀ 16 ♂)，每頭母羊之平均產仔數為 2.3 頭 (42/18)；而使用腹腔鏡人工授精方式授精 28 頭母羊，懷孕率為 71% (20/28)，母羊的總產仔數為 40 頭 (18 ♀ 22 ♂)，每頭母羊之平均產仔數為 2.0 頭 (40/20)。本試驗之結果顯示，以傳統人工授精或腹腔鏡人工授精對母羊懷孕率、總產仔數與每頭母羊之平均產仔數均無明顯之差異。

關鍵詞：腹腔鏡、人工授精、山羊。

緒言

山羊人工授精的實施，可加速母羊群的遺傳改良，節省種公羊飼養費用，避免近親配種與引種問題，加速後裔測定與選拔工作等。但是，傳統人工授精仍有其應用上限制存在，例如增加母羊發情觀察的人力、需要熟練的操作技術及受胎率較自然配種低等問題。歸咎造成受胎率及產仔數較低原因，可能與輸卵管內有效授精力之精子數目有關，有些母羊於發情階段子宮頸仍維持狹窄內徑，並有天然屏障阻礙精子授精。Killen and Caffery (1982) 發展腹腔鏡人工授精 (laparoscopic artificial insemination, LAI) 技術，避開母羊子宮頸複雜之構造，且能達到較佳受胎率之表現，而此技術已應用於許多品種，包括綿羊 (Robinson *et al.*, 1989; Scudamore *et al.*, 1991)、山羊 (Ritar and Ball, 1991) 與雪貂 (Wildt *et al.*, 1989; Howard *et al.*, 1991) 等，目前山羊之腹腔鏡人工授精尚未應用於國內之相關研究。因此本研究主要在建立山羊之腹腔鏡人工授精，評估懷孕率、產仔數，並與傳統人工授精進行比較。

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材料與方法

I. 試驗材料

選用 58 隻年齡約 2 至 5 歲之雜種母羊，平均胖瘦度評分爲 3 (1 =瘦弱，5 =肥胖)，分別施行傳統人工授精或腹腔鏡人工授精技術。

II. 試驗方法

(i) 母羊發情同期化處理

第 0 天(母羊發情當日)在母羊陰道內置入陰道助孕素塞劑 CIDR[®](controlled internal drug release; CIDR, EAZI-BREED[™], Australia)，第 9 天肌肉注射 400 IU PMSG 及 50 μ g PGF_{2 α} ，第 11 天移除 CIDR，移除後 30 小時，觀察母羊之發情。母羊之發情偵測採個別觀察，而發情穩定之母羊於 24 小時內分別完成人工授精。

(ii) 精液處理

冷凍精液處理參考 Evans and Maxwell (1987) 之方法，即冷凍精液取出後放入 37℃ 溫水中解凍 30 秒，解凍後之冷凍精液裝置於人工授精注入器內，而存活率低於 60%、畸形率高於 20%，均不宜供爲人工授精使用。

(iii) 腹腔鏡人工授精方式

試驗羊隻在施行腹腔鏡人工授精前，應至少禁食 24 hr。進行腹腔鏡人工授精時，採取頭低腳高傾斜 30 度的姿勢，其步驟參考 Armstrong and Evans (1984) 及 Robinson *et al.* (1989) 之方法，並略作某些修正。以 250mg ketamine 進行麻醉，將套管針 (Laparoscopic trocar) 置於套管 (trocar sleeve) 內，並於腹中線附近皮膚切開第一個小洞後插入，然後移除套管針放入腹腔鏡鏡頭，經由鏡頭探視腹腔。導氣孔連接自動充氣機自腹部灌注 CO₂，藉由光源避開血管於腹中線切開第二個小洞，利用儀器進行確認卵巢與子宮角之位置，隨後置入人工授精注入器 (IMV, L'Aigle, France) 使穿透管腔，使冷凍精液受精之位置於子宮角 (圖 1、2)，人工授精後靠近皮膚之切開傷口使用 Michel[®] 傷口夾 (Miltex Instrument Co., Germany) 夾合處理。

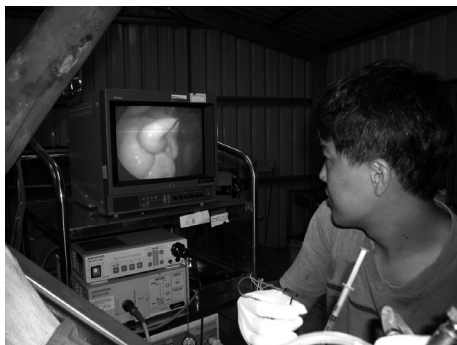


圖 1. 技術員操作穿透子宮角之位置。

Fig. 1. The technician punctures the uterine horn.



圖 2. 精液直接注入子宮管腔內。

Fig. 2. The semen is injected directly into the lumen of the uterus.

(iv) 傳統人工授精之步驟

傳統人工授精之步驟參考 Chemineau *et al.* (1991) 及 Leboeuf (2000) 之方法，即母羊先行固定，使傾斜呈 45 度向前站立，先用酒精棉擦拭乾淨母羊之外陰部，然後自陰道溫和緩慢的插入開腔器，以開腔器遭遇輕度阻力為止。打開開腔器的燈源或頭燈，壓開腔器的握把，以張開母羊陰道，再以光源照射陰道，檢視陰道內粘液狀況，並尋找子宮頸口位置。一般需經過子宮頸 1 至 5 道環後，注入器前端才能到達子宮體的位置，然後將精液緩慢(約 3 至 5 秒)注入子宮體的位置，再取出人工授精注入器及開腔器。在人工授精後第 45 天，藉由超音波妊娠診斷儀器檢查所有羊隻是否懷孕。

III. 試驗結果之統計分析

試驗所得資料利用 SAS(statistical analysis system, SAS 9.1, 2005)進行統計分析，並以 T-Test 比較兩組之平均值差異，顯著水準為 $P < 0.05$ 。

結果與討論

山羊為季節性生殖動物，其發情亦呈現一定的季節性變化。由於發情同期化之效果，除受藥品及其劑量的直接影響外 (Robinson, 1965; Robinson *et al.*, 1967; Cognie and Mauleon, 1983)，亦與羊隻品種、個體差異，以及季節、氣候等諸多因素有關 (Freitas *et al.*, 1997; Romano *et al.*, 1997; McKelvey, 1999)。本實驗將試驗羊隻以發情同期化處理，然後分別施行傳統人工授精或腹腔鏡人工授精。

在自然狀態下，羊群中個別羊隻發情的時間是不一致的。正在發情之羊隻處於濾泡期，而大部份不發情的羊隻則處在黃體期的早、中、晚等不同時期。發情同期化之處理，就是要使羊隻在預定的時間同時處於發情狀態。本實驗後續進行人工授精實驗，必須將發情同期化不一致狀況排除，故實施山羊發情同期化處理比較，結果列示於表 1。由表 1 之結果顯示不同組之發情率，並無顯著差異，因此再進行後續人工授精實驗。

表 1. 山羊腹腔鏡人工授精及傳統人工授精之同期化發情比較

Table 1. Estrus synchronization between laparoscopic and conventional artificial insemination of goats

Method	No. of goats	No. of goats Responded (%)
Conventional AI	36	30/36 (83%)
Laparoscopic AI	35	28/35 (80%)

發情同期化技術以及隨後的發情觀察記錄是人工授精的重要技術環節之一 (Romanao, 1996)，Moore and Shelton (1964) 最早建立供胚與受胚羊發情同期化之步驟。之後研究發情同期化，使用孕酮素 (Progesterone) 處理供胚與受胚綿羊 (Shelton and Moore, 1966)，Corteel (1973, 1975) 為提升人工授精受胎率，研究如何能更精確控制母羊發情時間。目前文獻上發情同期化較被廣泛使用 CIDR[®] 合併垂體激素與 PGF_{2α} 處理 (Moore and Epplenton, 1979; Ishwar and Pandey, 1990; Fonseca *et al.*, 2002)，與本實驗採用方法一致。

人工授精是種羊場配種的必備方法，該技術可提高種羊的配種效率，擴大配種母羊的頭數，加速肉羊雜交改良促進育種改良，降低飼養管理費用，防止各種疾病，特別是生殖道傳染病的傳播，提高母羊受胎率，擴大種公羊配種地區範圍。過去主要推廣傳統人工授精技術，其方法簡單，所需設備和技巧較少，但精液放置於陰道，受精機會相對降低。如果足夠的精液被放置在子宮頸內(~300百萬)，對懷孕之效果較佳，但目前國內施行傳統人工授精之結果並不一致，造成精液費用、勞力損失，故一般認為商業化之可行性降低。Killen and Caffery (1982) 發展腹腔鏡人工授精技術，避開羊隻複雜之子宮頸解剖構造，且能提供較佳之受胎率。國內目前尚未有此相關研究討論，故本試驗以傳統人工授精及腹腔鏡人工授精進行比較，結果列示於表 2。使用傳統人工授精方式之懷孕率為 60% (18/30)，母羊分娩之總產仔數為 42頭 (26 ♀ 16 ♂)，每頭母羊之平均產仔數為 2.3頭 (42/18)；另外使用腹腔鏡人工授精方式，懷孕率為 71% (20/28)，較傳統人工授精方式為高，而母羊分娩之總產仔數為 40頭 (18 ♀ 22 ♂)，每頭母羊之平均產仔數為 2.0頭 (40/20) (圖3)，但均與傳統人工授精方式在統計上無顯著差異。

表 2. 山羊腹腔鏡人工授精及傳統人工授精方式之比較

Table 2. Comparisons between laparoscopic and conventional artificial insemination of goats

Method	No. of goats	Pregnancy rate (%)	No. of kids	Average litter size (kids/doe kidding)
Conventional AI	30	60(18/30)	42(26 ♀ 16 ♂)	2.3(42/18)
Laparoscopic AI	28	71(20/28)	40(18 ♀ 22 ♂)	2.0(40/20)



圖 3. 以腹腔鏡人工授精生產四胞胎仔羊。

Fig. 3. Quadruplets were born by laparoscopic artificial insemination.

過去研究山羊腹腔鏡人工授精可有效改善受胎率 (Moore *et al.*, 1988 ; Ritar *et al.*, 1990 ; McKelvey, 1999)，比較山羊腹腔鏡人工授精與傳統人工授精之受胎率分別為 62.6% 和 49.3% ($P < 0.05$)，統計呈現顯著差異 (Vallet *et al.*, 1992)。目前國外使用腹腔鏡人工授精，羊隻之懷孕率為

48% (Windsor *et al.*, 1994) 至 72% (Hill *et al.*, 1998) 的範圍間。本實驗使用腹腔鏡人工授精之懷孕率已有提升，但懷孕率、每頭母羊之平均產仔數，在統計上並無顯著差異，未來可進一步探討之間差異。

腹腔鏡人工授精之另一優點為精液之使用量相對較少，授精過程大約5分鐘，且經由熟練的操作員執行，母羊術後良好，亦不影響日後重覆施行之效果 (Armstrong and Evans, 1984)，但是由於需專業設備、技能與特殊藥物，且必須有經驗技術員操作，所需費用較為昂貴。有些研究顯示，不同品系羊隻間施行傳統人工授精之受胎率有顯著差異，但使用腹腔鏡人工授精之受胎率並無品種差異，且可相對提升受胎率 (Fair *et al.*, 2005)。近年來，於其他品系羊隻之非傳統人工授精技術研究，也有相當具體之成果 (King *et al.*, 2004; Anel *et al.*, 2005; Fair *et al.*, 2005; Sohnrey and Holtz, 2005)。

繁殖效率是影響羊隻飼養效益的重要因素之一，而提高繁殖效率及產仔率是為養羊戶的共同追求。山羊腹腔鏡人工授精技術，未來可再進一步將授精精子數降低，並達成最佳懷孕率，以加強品種改良之效益，提升產業競爭力，使養羊事業永續發展。

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A study on the laparoscopic artificial insemination of goats ⁽¹⁾

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Abstract

This experiment was conducted to evaluate the effects of laparoscopic artificial insemination and conventional cervical insemination on the pregnancy rates, the total kidding number and the average litter size. Goats with estrus synchronization were inseminated with conventional cervical and laparoscopic intrauterine artificial insemination. The pregnancies were verified by ultrasonic scanning at 45 days after insemination. Thirty goats were inseminated with conventional cervical artificial insemination, the pregnancy rate was 60% (18/30), the total kidding number was 42 (26 ♀ and 16 ♂) and the average litter size was 2.3/doe (42/18). Twenty-eight goats were inseminated with laparoscopic artificial insemination, the pregnancy rate was 71% (20/28), the total kidding number was 40 (18 ♀ and 22 ♂) and the average litter size was 2.0/doe (40/20). There were no significant differences in the pregnancy rate, the total kidding number and the average litter size between conventional artificial insemination and laparoscopic artificial insemination.

Key words: Laparoscopy, Artificial insemination, Goat.

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