

山羊超級排卵最適條件之探討⁽¹⁾

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摘要

本研究主要建立山羊有效超級排卵方式，試驗一評估雜交種與純種努比亞山羊的超級排卵反應，以雜交種母羊與純種母羊各 12 頭，分別進行超級排卵處理，在發情後第 7 日，採用腹中線剖腹術進行羊胚之沖出及回收，檢視其卵巢反應。試驗結果顯示相同超級排卵處理方式，所得平均黃體數、胚回收數及可用胚數等均以雜交種山羊顯著較純種努比亞母羊為高 ($P < 0.05$)。試驗二探討在激性腺素 (gonadotrophin) 超級排卵處理之前列腺素 (prostaglandin, $\text{PGF2}\alpha$) 注射時間， $\text{PGF2}\alpha$ 在以激性腺素超級排卵處理時分三組，使用純種努比亞母羊共 36 頭，第一、二及三天分別注射 $\text{PGF2}\alpha$ 後評估母羊卵巢之反應。試驗結果顯示在超級排卵處理之第三天注射 $\text{PGF2}\alpha$ ，平均黃體數，胚回收數及可用胚數，均顯著 ($P < 0.05$) 高於超級排卵處理之第一、二天注射者。

關鍵詞：山羊、超級排卵、激性腺素。

緒言

台灣本地山羊有體型小、生長速度慢、屠宰率低、經濟效益差等問題 (蘇, 2003; 吳等, 2005)。為增加養羊業的經濟效益，擴大本省優良羊種的族群，曾先後自國外引進努比亞山羊與波爾山羊等品種進行改良，但是由於自國外引進種羊手續繁雜，費用較高，週期較長，且單靠進口種羊的自然繁殖來改良本地羊隻，又往往不能滿足本省養羊事業發展的需要。因此應用同期發情、超級排卵及胚胎移植等技術，以擴充純種羊隻之族群，是解決此一問題的有效途徑之一。

羊隻超級排卵之成效，受到許多方面之因素所影響，根據過去本場之研究數據顯示，羊隻經超級排卵處理後，所收集到可用胚之數目少者為零個，即卵巢無反應，而最多可達四十幾個，其個體差異相當大。使用外源激素誘發多濾泡發育的效果不一，且個別羊隻對激性腺刺激之敏感性差異很大，而濾泡的生長、成熟與排卵均由激濾泡素 (follicular stimulating hormone, FSH) 和排

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卵素 (luteinizing hormone, LH) 的分泌模式所調控, 又家畜個體間腦下腺所分泌的 FSH 和 LH 的比例亦有差異 (Driancourt *et al.*, 1990 ; Herrler *et al.*, 1991 ; Taft *et al.*, 1996 ; Gonzlez-Bulnes *et al.*, 2004)。因此相對使羊隻的發情持續時間、排卵時間亦各異, 因此需要探討品種間之差異。另外, 並以不同 PGF2 α 注射時間合併激性腺素超級排卵處理, 探討卵巢之反應。在動情週期不同階段注射 PGF2 α 後, 可有效誘導黃體退化, 而使多數母羊回復至發情狀態 (Acritopoulou and Haresign, 1980), 但母羊個體間之反應呈現顯著差異, 影響發情同期化與人工授精排卵時間之掌控, 內分泌之運用性, 其反應差異之原因可能與 PGF2 α 之注射時間不同致影響卵巢之反應有關 (Houghton *et al.*, 1995)。本研究評估雜種母羊與純種母羊對超級排卵處理之反應, 並選用純種努比亞母羊探討在超級排卵處理過程中 PGF2 α 之注射時間, 對黃體數目、胚回收數目及可用胚數目的影響。

材料與方法

I. 實驗設計

試驗一評估雜交種與純種努比亞山羊的超級排卵反應, 以雜種母羊與純種母羊各 12 頭, 分別進行相同超級排卵處理, 在發情後第 7 日, 採用腹中線剖腹術進行羊胚之沖出及回收, 檢視其卵巢反應, 如平均黃體數、胚回收數及可用胚數等。試驗二分為三組, 其使用純種努比亞母羊共 36 頭, 探討在激性腺素 (gonadotrophin) 超級排卵處理後之第一、二及三天分別注射 PGF2 α , 然後評估母羊卵巢之反應, 如黃體數、胚回收數及可用胚數。

II. 供胚母羊之超級排卵

以肥瘦度良好, 且有正常生殖功能的母羊為供胚母羊。試驗羊群以本場雜種肉羊及純種努比亞山羊為供胚者, 並依試驗分組。母羊動情週期之調節係採用陰道助孕素塞劑 CIDR[®] (controlled internal drug release; CIDR, EAZI-BREEDTM, Australia) 留置 11 日, 在 CIDR[®] 留置後第 9~11 日間, 每隔 12 小時以遞減劑量方式, 肌肉注射 6 劑, 總量為 20 A.U. (Armour units) 由豬腦下腺萃取之 FSH (follicle stimulating hormone from porcine pituitary, pFSH, 川崎製藥株式會社, 日本) 與總量為 6 單位劑量之 LH (luteinizing hormone from ovine pituitary, LH, Sigma, U.S.A) 進行超級排卵。另外, 並配合試驗處理時間, 分別注射 1 ml Prosolvin (luprostiol 7.5 mg/ml 合成之 PGF2 α ; Prosolvin[®], Intervet, Holland)。待母羊發情後, 每隔 12 小時以同一品種公羊進行自然交配一次, 直至發情結束為止。

III. 胚胎收集

供胚母山羊於發情後第 7 日(發情日為第 0 日), 採用腹中線剖腹術進行羊胚之沖出及回收。並檢視其卵巢狀態, 以瞭解各種不同處理對母山羊黃體數、胚回收數、可用胚數、濾泡數、白體數之影響。胚之收集與評估, 是根據 Nuti *et al.* (1987) 之研究方式, 將所有胚分別以倒立顯微鏡鏡檢, 以檢查具發育階段和品質(外觀及形態特徵)。

IV. 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) 比較組間平均值差異之顯著性。

結果與討論

評估雜種肉羊與純種努比亞山羊的超級排卵反應，結果列於表 1。由表 1 之結果顯示，進行超級排卵之雜種母羊的平均黃體數為 13.0 ± 1.8 、胚回收數為 10.3 ± 1.5 、可用胚數為 9.6 ± 0.6 ，而純種努比亞母羊組之平均黃體數為 6.0 ± 1.2 、胚回收數為 5.0 ± 0.8 、可用胚數為 4.0 ± 0.6 ，故以雜種母羊之超級排卵效果顯著優於純種母羊 ($P < 0.05$)。

表 1. 激性腺素處理對雜交種與努比亞母羊超級排卵之反應

Table 1. Effect of gonadotropin treatments on superovulatory response between Crossbred and Nubian goats

Parameters	Crossbred goats	Nubian goats
No. of does	12	12
Corpora lutea /does	13.0 ± 1.8	6.0 ± 1.2^a
Recovered embryos/does	10.3 ± 1.5	5.0 ± 0.8^a
Viable embryos/does	9.6 ± 0.6	4.0 ± 0.6^a
Follicles/does	11.3 ± 2.7	4.5 ± 1.2^a
White avascular corpora/does	2.5 ± 0.5	5.0 ± 0.2^a

^a Mean in the same row differ significantly ($P < 0.05$).

超級排卵的原理是在動物發情週期的特定階段，以外源內泌素處理，提高血液中激性腺素之濃度，降低發育濾泡的閉鎖率，增加濾泡發育到成熟的數量。超級排卵為一複雜之生理過程，至今其效果仍不夠穩定，特別是個體之間差異很大 (Whyman and Moore, 1980; Evans and Armstrong, 1984; Moor *et al.*, 1984; Hawk *et al.*, 1987; Goulding *et al.*, 1990; Guilbault *et al.*, 1991)，歸納其原因可分為外在因素如超級排卵處理步驟及外源內泌素種類 (Nowshari *et al.*, 1992) 或內在因素如基因 (Bindon *et al.*, 1986; Nuti *et al.*, 1987)、品種、年齡 (Morre, 1982)、營養、繁殖適期 (Baril *et al.*, 1993; Cognie, 1999; Driancourt, 2001)，其中以品種差異為主要之影響因素 (Bindon *et al.*, 1986; Torres *et al.*, 1987)。據 Vivanco *et al.* (1994) 之研究，在 9000 頭綿羊之超級排卵試驗中，品種之對胚生產之影響幾乎佔 30%，而亦有研究認為超級排卵效果之差異可能源自於遺傳因素 (Bondurant *et al.*, 1986)，故建立不同品種間可信賴之超級排卵處理步驟則相當重要。

羊隻品種會影響濾泡生長及排卵率 (Bindon *et al.*, 1986)，而不同品種所造成胚生產之差異，可能與濾泡生產形式、濾泡動力學、濾泡對 FSH 處理之反應有關 (Fry *et al.*, 1987; Picton and McNeilly, 1991; Ammoun *et al.*, 2006)。使用外源激性腺素作超級排卵處理時，控制濾泡生長和卵巢內之作用機制，將會影響排卵率及胚回收數 (Adams *et al.*, 1988; Goulding *et al.*, 1990; Guilbault *et al.*, 1991; Cognie, 1999)，而導致超級排卵之反應結果不同 (Torres and Cognie, 1984)，故個體間之差異明顯。

母羊於動情週期中，血液生殖相關內分泌濃度之變化，特別是不同生殖內分泌濃度間的比例與濾泡發育成熟及排卵間之關係，為繁殖生理領域中之研究重點。Campbell *et al.* (1995) 認為在濾泡的發育過程中，FSH 佔至為關鍵地位，即 FSH 與原始濾泡的恢復發育有關，而 LH 可能在濾泡的選擇發育上，即濾泡優勢化上起關鍵作用。因此 FSH/LH 之適當比率對於卵巢反應上具重要的角色 (Moor *et al.*, 1984; Lindsell *et al.*, 1986; Donaldson, 1990; Henderson *et al.*, 1990; Picton *et al.*, 1990; Cognie, 1999)。在綿羊之試驗中，FSH 之製劑內含低量 LH，故其超級排卵反應之效果較佳 (Armstrong and Evans, 1994)，此與牛方面之研究結果一致 (Herrler *et al.*, 1991)。在某些品種牛隻以較低 FSH/LH 之比率處理，會使排卵數量增加 (Chupin *et al.*, 1985)。由本研究之結果顯示使用相同方式之超級排卵，結果於雜種母羊與純種母羊間呈顯著差異 ($P < 0.05$)，因此未來可進一步建立品種間可信賴之超級排卵處理步驟，並探討超級排卵對不同品種之 FSH/LH 之適當比率。

PGF2 α 經常使用於動物之發情同期化及超級排卵，而被視為影響黃體退化之因素 (McCracken *et al.*, 1972; Armstrong *et al.*, 1983)。注射 PGF2 α 產品於母羊群動情週期之任何階段，可導致多數母羊之黃體退化，而恢復至發情起始時期 (Acritopoulou and Haresign, 1980)。但是母羊對 PGF2 α 處理之反應顯現極大之差異，以致影響發情同期化與人工授精時間點之掌控，而造成反應差異之原因為可能與 PGF2 α 處理時間點及母羊卵巢之發育階段有關。PGF2 α 於週期中之注射時間會影響發情之起始 (Houghton *et al.*, 1995)，而通常在黃體期注射 PGF2 α 才有效果。以往我們對超級排卵之處理並未考慮 PGF2 α 之處理時間，因此本試驗在超級排卵處理之第一、二及三天分別以 PGF2 α 處理，評估母羊卵巢的反應，以了解對母山羊黃體數、胚回收數、可用胚數之影響。

本試驗之結果，列示於表 2。在超級排卵處理後之第三天，注射 PGF2 α 之平均黃體數為 20.0 ± 2.2 、胚回收數為 17.0 ± 1.5 、可用胚數為 15.0 ± 1.3 ，均顯著高於在超級排卵處理後之第一與二天注射 PGF2 α 組 ($P < 0.05$)。在注射 PGF2 α 後，黃體解體需要一段時間，而且母羊對 PGF2 α 的反應也存在一些差異，導致有些黃體解體速度較快，有些黃體溶解速度較慢，因此發情並不是很集中。PGF2 α 注射時間會影響發情起始及間距 (Ishwan and Pandey, 1990)，其原因與濾泡波之起始具相關性 (Viñoles and Rubianes, 1998; Duggavaathi *et al.*, 2004, 2005)。據研究顯示，PGF2 α 注射後，有些母羊呈現不反應，其原因可能與注射時間在黃體之早期階段有關，乃因為新形成之黃體，會影響 PGF2 α 之作用 (Acritopoulou and Haresign, 1980; Barrett *et al.*, 2002)。有研究藉由超音波觀察卵巢之反應，結果發現在超排激性腺素注射第三天再施打 PGF2 α ，可有效導致週期迅速開始，並造成助孕素含量急劇降低 (Rubianes *et al.*, 1997a,b)。PGF2 α 注射時間合併激性腺素進行超級排卵處理，當 PGF2 α 投用與 FSH 第 5、6 次之注射(即第 3 天處理)，檢視卵巢之反應，黃體分解率高達 85% (Rubianes *et al.*, 1997a,b)。Rubianes (2003) 之研究顯示，母羊超級排卵處理第三天的黃體，投用 PGF2 α 對黃體解體作用極為敏感，於排卵發生 48 至 72 小時投用 PGF2 α ，造成助孕素含量急劇降低。使用 PGF2 α 於超級排卵處理第一天並無反應 (Acritopoulou and Haresign, 1980)，相似之結果也見於牛之研究 (Rowson *et al.*, 1972)。目前努比亞山羊的超排效果，以在超級排卵激性腺素處理之第三天注射 PGF2 α 之效果最佳，此結果可提供未來在努比亞山羊超級排卵處理之應用，並利用胚胎移植技術擴大優秀努比亞的族群，以加速地方品種的改良。

表 2. 不同 PGF2 α 處理時間對超級排卵母羊卵巢反應之影響Table 2. Effect of different PGF2 α administration time on the ovarian response of superovulated does

Parameters	D1	D2	D3
No. of does	12	12	12
Corpora lutea /does	6.4 \pm 0.6 ^a	5.0 \pm 2.2 ^a	20.0 \pm 2.2 ^b
Recovered embryos/does	5.2 \pm 0.5 ^a	4.0 \pm 1.2 ^a	17.0 \pm 1.5 ^b
Viable embryos/does	4.0 \pm 0.3 ^a	3.0 \pm 0.6 ^a	15.0 \pm 1.3 ^b
Follicles/does	4.2 \pm 0.8 ^a	3.2 \pm 0.4 ^a	1.0 \pm 0.2 ^b
White avascular corpora/does	3.8 \pm 0.8 ^b	14.0 \pm 1.5 ^a	0.8 \pm 0.3 ^{bc}

^{a,b,c} Means in the same row with different superscripts differ significantly ($P < 0.05$).

D1, 1 ml synthetic PGF2 α (Prosolvin® , Intervet , Holland) treatment at the first day of gonadotropin injections; D2, 1 ml synthetic PGF2 α treatment at the second day of gonadotropin injections; D3, 1 ml synthetic PGF2 α treatment at the third day of gonadotropin injections.

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Investigations of the essential factors for optimal superovulation in goats ⁽¹⁾

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Abstract

This study was conducted to investigate the essential factors affecting superovulation of goats in order to establish the superovulation methodology. In experiment I, the differences of superovulatory response were compared in Crossbred goats and Nubian goats. This experiment was conducted with twelve goats of each breed. Seven days after mating, the embryos were flushed and recovered through the aid of mid ventral laparotomy and ovarian response was recorded. The results showed that the number of corpora lutea/does, recovered embryos/does and viable embryos/does of crossbred does were significantly higher than those of Nubian does ($P < 0.05$). In experiment II, PGF2 α were administered at different time after gonadotrophins injection for superovulation. Thirty-six Nubian goats were randomly divided into three equal groups. Ovarian responses were evaluated following PGF2 α administrated at the time of the first, second or third day gonadotrophins injection for superovulation. The number of corpora lutea, recovered embryos and viable embryos of PGF2 α treatment at third day group were significantly higher than those in the first or second day treatment group ($P < 0.05$).

Key words : Goat, Superovulation, Gonadotrophin.

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