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**THE ROLE OF PLASMA PROLACTIN CONCENTRATION IN  
SEASONAL FIBRE GROWTH CYCLES IN DOWN-PRODUCING  
GOATS AND WILTSHIRE SHEEP**

**A THESIS PRESENTED IN PARTIAL CULFILMENT OF THE REQUIREMENTS FOR THE  
DEGREE OF DOCTOR OF PHILOSOPHY IN ANIMAL SCIENCE AT MASSEY  
UNIVERSITY**

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**1996**

*The effort involved in this thesis is dedicated to my children Keshia and Ryan Melton who tolerated their working mother and fed the animals and never asked why.*

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## ERRATA

Insert as page 121b:

The radioimmunoassay of PRL was conducted using ovine PRL (NIDDK-oPRL-I-2) for standards and radiiodination, and ovine PRL antiserum (NIDDK-anti-oPRL-2). PRL was iodinated by the Iodogen technique (Pierce, Rockford, IL) using [125I]-iodide (New England Nuclear NE0033A). Separation of antibody-bound from free labelled PRL was by second antibody precipitation using excess goat antirabbit serum (SAR 265 generated at Ruakura Agricultural Centre). The assay was validated for caprine samples. Sensitivity was 0.6 ng/ml and assay range was up to 100 ng/ml. Intra-assay and inter-assay coefficients of variation at 32 ng/ml PRL concentration were 12.1% and 14.4% respectively.

<b>Page</b>	<b>Line*</b>	<b>As Written</b>	<b>Should be</b>
(*Line refers to line of actual text)			
ii	13	hours by	hours
v	24	increase	increased
vi	25	decline	declined
vii	4	fib re	fibre
vii	8	plasm	plasma
vii	21	61 ml/min	6 ± 1 ml/min
vii	25-26	infusates established ...	and PRL infusates were sterile:
xv	Fig 2.1	length	length
xix	Fig 6.1	igoatnervals	intervals
5	27	ends, can	ends which can
12	last	females goats	female goats
15	16	dieing	dyeing
18	Fig 1.10	nixon	Nixon et al., 1991
20	11	as it is under	delete "as it is under"
20	18	Slee	(Slee
22	5	Similarly the	delete "the"
29	12	inhybition	inhibition
29	22	this	This
31	21	lactatrophs	lactotrophs
42	20	imnuroreactive	immunoactive
43	2	concentration	concentration were
44	7	quiesense	quiescence
50	1	have been conducted	delete "have been conducted"
51	19	gland and Other	gland. Other
51	last	keratinocycle	keratinocyte
53	2	chormone	hormone
61	11	Polactin	Prolactin
61	13	goats,	delete "goats"
61	16	Person	Pearson

66	19	)impaired	) are impaired
66	23	cells and	cells
66	19	)impaired	) are impaired
68	21	apparent	is apparent
70	15	PhD	PhD study
99	5	downe	down
101	Y axis, Fig 2.11	Proanagen Secondary	SAC+brush
102	last	represnet	represent
105	Table 2.15	DGR	fibre diameter
		mg/cm2/day	micron
109	21	80 micron, down	80 micron and down
115	5	in sheep, a a peak	in sheep, a peak
115	18	increase plasma	increase in plasma
118	2	PRL increase	PRL
118	17	cycle shorter	cycle is shorter
119	2nd	last events are	events is
121	11	outlined in Section 2.3.2.3	outlined on Page 121b
124	1	11 January	1 January
131	13	timing ... were	timing ... was
134	5	anager	anagen
138	11	mealtonin	melatonin
141	7	but secondary	not secondary
148	4	Section 2.3.2.1	delete "Section 2.3.2.1"
	12	Section 2.2.3.2	Section 2.3.3.2
	17	Section 2.2.3.3	Section 2.3.3.3
165	15	pars distalis	pars distalis
166	last	effects treatment	effects of treatment
167	3rd last	suppliers were	suppliers was
169	last	replicated time	replicated in time
173	6	1.0 ± 0.1	1.0 ± 0.1 hour
175	3	determined 168 hours	determined at 168 hours
175	7	caluclate	calculate
176	(infusion 3)	- 168	168
177	Fig 5.4	mg/sheep	mg/sheep)
177	2nd last	PRC	PRL
177	2nd last	sleep	sheep
178	5	Suprisingly	Surprisingly
184	9	charges	changes
184	11	endgenous	endogenous
184	last	was compromised	compromised
188	7	spring rise	spring rise in

190	6	occur	occurs
191	8	regimes	regimens
196	9	increased	increasing
197	Fig 6.1	igoatintervals	intervals
208	8	gpats	goats
213	10	immunoactive	immunoreactive
213	16/17	values in high levels	high levels
216	8	called	term
217	2	proportion	proportion of follicles
217	5	emergance	emergence
217	24	induced in	induced changes in
219	17	fluctuations can	can
220	12	ng/ml goats	ng/ml
220	13	LDBR	BRLD
222	last	iOn	in
224	16	follicles, plasma	follicles, changes in plasma
224	last	that	the
227	9	a	an
236	10	period,	period in SD goats,
236	11	in individuals goats fluctuated	in individuals goats PA fluctuated
241	21	31 March it	31 March
261	19	accound	account
262	4	occasions, treated	occasions, been treated
266	8	has found	was found
267	last	telogen goat follicles	telogen follicles of goats
268	13	In	in
268	19	impotence	importance
270	12	immunoactive	immunoreactive
275	19	source	source or
281	8	Table 8.2	Table 8.1
281	11	administer	administered
312	3	years, experiments	years' experiments
313	11	elicit	elicit
321	23	independently to of	independently of



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## ABSTRACT

This study examined the role of plasma PRL concentration in regulating seasonal fibre growth following the transition from short to long day photoperiod. In three down goat genotypes higher proportions of Angora genes extended the duration of guard hair growth, decreased biannual down growth and reduced the period of secondary follicles inactivity. The timing of follicle reactivation in spring and seasonal changes in plasma PRL concentrations were similar in all genotypes. Plasma PRL concentration increase, in spring, was associated with primary, but not secondary, follicle reactivation. Secondary follicle reactivation produced down of less than 2 mm which was associated with the shedding of winter down. Plasma PRL concentrations were suppressed, in spring and long-photoperiods (16L:8D), by injections of 1-5 mg/goat/day of bromocryptine and 2-3 weekly injections of long-acting bromocryptine (Parlodel). Injections of 1-5 mg/goat/day of domperidone elevated plasma PRL concentrations for 12 hours by and shedding was advanced. The circulating half-life of PRL, in sheep and goats, was  $42 \pm 6$  and  $104 \pm 14$  minutes following PRL injection or constant infusion respectively. In down goats, the normal spring-rise in plasma PRL concentration was suppressed using Parlodel or advanced by long day photoperiod. Increased plasma PRL concentration in spring provided anagenic signals to telogen primary and secondary follicles and catagenic signals to anagen secondary follicles. Following a reversal from short to long photoperiod anagen follicles of both goats and sheep entered telogen. Shedding occurred when the follicles subsequently reactivated. The suppression of plasma PRL concentration using Parlodel, during long photoperiod reversal, prevented the catagenic effect of long-photoperiod on anagen Wiltshire sheep follicles. In goats, however bromocryptine did not prevent follicles entering catagen but delayed follicle reactivation. The intravenous infusion of PRL had no effect on fibre growth in down goats or Wiltshire sheep. While the direct infusion of PRL to the skin caused an extreme local tissue reaction. Plasma PRL concentration has a role in regulating seasonal fibre growth cycles in down-producing goats but it is not a simple causal relationship and is dependent on follicle growth stage.

## SUMMARY

**Chapter 1:** The literature on fibre growth cycles in down and Angora goats and shedding sheep breeds was reviewed. Literature was presented on the regulation of plasma PRL concentration with special emphasis on seasonal factors regulating pituitary PRL secretion. The effects of plasma PRL concentration on fibre were also reviewed.

**Chapter 2:** Mixed-aged breeding does were categorised as having either no known Angora ancestry (F), a maximum of 25% Angora ancestry (C), or 50% Angora ancestry (G). The three genotypes were tested for differences in the quantity and timing of growth from primary and secondary follicles and seasonal plasma PRL concentrations. The sequence of primary follicle growth was similar in all three goat genotypes but the duration of growth of the guard hair fleece was 50-70 days longer in G compared to F goats. The timing of down growth was similar in all three goat genotypes. However, SA during July and August of 1991 and 1992 was more than 25% lower ( $P < 0.05$ ) in F goats compared to C or G goats. Summer down growth was identified in 78% of G, 97% of C and 100% of F goats. Plasma PRL concentrations were similar in all genotypes. It was concluded that, the inclusion of additional genes of the less seasonal Angora, extended the duration of growth from primary follicles and decreased biannual down growth and the expression of catagen in secondary follicles.

**Chapter 3:** In this experiment, the timing and correlation of fibre growth events and plasma PRL concentration events were determined for individual down-producing goats from July to February in natural photoperiod. PA began to increase on 17 September  $\pm 7$  days and primary follicles reached full activity on 1 January  $\pm 10$  days. Similarly, the mean date of secondary follicle activation was 17 September  $\pm 11$  days and rose to a summer peak of 38  $\pm 7\%$  on 31 October  $\pm 9$  days.

The increase in SA which eventually produced the winter down fleece commenced on 5 November  $\pm$  9 days. The date of primary follicle reactivation in spring was associated with the date at which plasma PRL concentration increased above 20 ng/ml ( $r=0.78$ ,  $P<0.01$ ). Associations between secondary follicle reactivation and plasma PRL concentration could not be found. In conclusion, it was found that the timing of maximum summer down SA was closely associated with maximum down fleece shedding and not associated with plasma PRL concentration.

**Chapter 4:** The effects on plasma PRL concentrations in down-producing goats of summer treatment with bromocryptine mesylate (BR) and spring and summer treatment with domperidone (DOM) were examined in two experiments. In experiment 1 (December), daily injections of BR (1-5 mg/goat/day) reduced mean plasma PRL concentration to less than 12 ng/ml compared with control concentrations of  $87 \pm 7$  ng/ml ( $P<0.001$ ). A single BR injection suppressed plasma PRL concentration for 20 hours ( $P<0.05$ ). DOM injections (1-5 mg/goat/day) increased mean plasma PRL concentrations to between 278 and 548 ng/ml for 12 hours. In experiment 2 (September), plasma PRL concentrations following DOM administered at 2.5 mg/day for 14 days either by subcutaneous injection (DOMinj), or by a subcutaneously fitted osmotic minipump (DOMosp) were  $612 \pm 32$ ,  $73 \pm 35$  and  $60 \pm 34$  ng/ml in DOMinj, DOMosp and control respectively ( $P<0.001$ ). Mean plasma PRL concentrations immediately prior to the injection were lower ( $P<0.01$ ) in DOMinj ( $33 \pm 6$  ng/ml) goats compared with DOMosp ( $72 \pm 7$  ng/ml) and control ( $60 \pm 8$  ng/ml) groups. Shedding was advanced in DOMinj goats compared with control goats. It was concluded that plasma PRL concentration in goats can be successfully manipulated using single injections of bromocryptine and domperidone, but the effect is transitory.

**Chapter 5:** The circulating half-life of PRL was determined in sheep and goats, and methods for manipulating plasma PRL concentration, by PRL infusion, long-acting bromocryptine, and long-photoperiod were determined for sheep.

In sheep and goats the  $T_{1/2}$  of PRL was  $42 \pm 6$  or  $105 \pm 14$  minutes following PRL injection or infusion respectively. In sheep,  $T_{1/2}$  was longer ( $P < 0.01$ ) when PRL was infused at 0.1 (P1;  $144 \pm 15$  minutes) compared to 0.4 (P2;  $55 \pm 13$  minutes) mg oPRL/kgLW/day. In PRL-infused sheep, mean treatment plasma PRL concentration was higher in P2 ( $99 \pm 17$  ng/ml), relative to P1 ( $60 \pm 17$  ng/ml) and C ( $59 \pm 17$  ng/ml) sheep ( $P < 0.05$ ). Plasma PRL concentration was suppressed to below 15 ng/ml in sheep for 21 days after the long-acting bromocryptine injection. Long photoperiod treatment increased mean plasma PRL concentration to LD sheep ( $170 \pm 33$  ng/ml). In conclusion, plasma PRL concentration could be manipulated in sheep by PRL infusion, long photoperiod and long-acting bromocryptine treatment.

**Chapter 6:** The effect on fibre growth of down goats of either delaying or advancing the spring rise in plasma PRL concentration increase was studied from late July to middle of October. Goats were: maintained in natural spring photoperiod and received no further treatment ( $n=10$ , C); were injected with long-acting bromocryptine ( $n=10$ , BR); or were treated with long photoperiod (16L: 8D), with no further treatment ( $n=5$ , LD), or were injected with long-acting bromocryptine ( $n=5$ , BRLD). Mean overall plasma PRL concentration, in comparison to C goats (27 (23-31) ng/ml), was higher in LD goats (87 (69-109) ng/ml,  $P < 0.001$ ) and lower in both BR (4.2 (3.5-5.0) ng/ml,  $P < 0.001$ ) and BRLD (9 (7-12) ng/ml,  $P < 0.01$ ) goats. The mean date when all primary follicles became active in C goats was 26 December  $\pm 8$  days, which was similar to that of BR (16 January  $\pm 8$  days) goats but later than the 1 November  $\pm 11$  ( $P < 0.001$ ) and 14 November  $\pm 11$  days ( $P < 0.02$ ) when maximum activity was achieved in LD and BRLD goats. Mean SA increase to be  $77 \pm 7\%$  at the termination of treatment in BR goats (C  $23 \pm 8\%$ ,  $P < 0.01$ ) while in LD goats SA increased to  $42 \pm 9\%$  after 41 days of treatment and then fell to be  $19 \pm 10\%$  at the end of the treatment period. Mean SA increased throughout the treatment period to reach  $59 \pm 11\%$  and  $77 \pm 7\%$  in BRLD and BR goats respectively at the end of the treatment period. At the termination of the bromocryptine treatment, SA decreased.

In BR goats, the early activation of secondary follicles was not associated with early summer down fleece emergence. The conclusion was that increased plasma PRL concentration provided anagenic signals to telogen secondary follicles and catagenic signals to anagen secondary follicles. The primary follicles, plasma PRL increase had an anagenic effect on telogen follicles.

**Chapter 7:** Following a reversal from short to long photoperiod the effects of suppressing the increase in plasma PRL concentration, using long-acting bromocryptine treatment, was studied from both down goats and Wiltshire sheep. Following 6 months pre-treatment short photoperiod down-producing does continued under short-photoperiod (SD goats, n=6), while Wiltshire sheep and goats were treated with long photoperiod (16L:8D) with either no further treatment (LD goats n=6, LD sheep n=6), or treatment with long-acting bromocryptine (BRLD goats, BRLD sheep) at two weekly intervals from 7 January until 31 March 1993 and then released onto pasture under natural photoperiod. Mean plasma PRL concentration during the treatment period was 6.0 (5.3-6.8 ng/ml), 8.5 (7.6-9.5) ng/ml and 97 (89-109) ng/ml in SD, BRLD and LD goats respectively ( $P < 0.001$ ) and 0.67 (0.74-0.87) ng/ml and 135 (123-148) ng/ml in BRLD and LD sheep respectively ( $P < 0.001$ ). In sheep compared to goats, mean plasma PRL concentration was 8 ng/ml lower during bromocryptine treatment ( $P < 0.0001$ ) and 38 ng/ml higher during long photoperiod treatment ( $P < 0.05$ ). During the treatment period mean PA and SA of SD goats remained high (PA 49-73%, SA > 90%). However in LD sheep and goats, after the reversal from short to long photoperiod SA and PA fell to below 40% on 3 March 1993 before returning to be in excess of 73% on 31 March 1993. In BRLD sheep, mean PA and SA remained above 86% throughout the treatment period. In BRLD goats, mean PA and SA decline to be less than 40% at the end of the treatment period. In comparison to LD goats, follicle reactivation was delayed in BRLD goats. It was concluded that there was evidence that plasma PRL concentration mediated the catagenic effect of long photoperiod on anagen Wiltshire sheep follicles but not in anagen down-producing goat follicles.

In down goats, plasma PRL concentration may have a role in mediating the anagenic effect of long-photoperiod on telogen goat follicles.

**Chapter 8:** Following pre-treatment with short-photoperiod, the effects on follicle and fibre growth of elevating plasma PRL concentration by either whole body or local skin infusions were determined. From 2 February 1994 to 3 March 1994, goats were treated with short photoperiod and either received no further treatment (n=5, SD) or an infusion of PRL (0.5 mg/kg<sup>0.75</sup>/day) via the jugular vein (n=5, P) or were treated with long photoperiod (n=5, LD). Mean plasma PRL concentration during the treatment period in SD (10 (8-13) ng/ml) goats was lower than in either LD (52 (42-72) ng/ml, P<0.01) or P (29 (23-36) ng/ml, P<0.05) goats. Mean PA reached minimal levels (21-30%) on 20 March in LD goats but not until 8 April in P and SD goats, with PA returning to levels in excess of 94% by 6 May in all treatments. The mean date of minimum SA in LD goats was 23 March±4 days which was earlier than in either P (10 April±4 days, P<0.01) or LD (6 April±4 days, P<0.05) goats. By 6 May 1994, mean SA in all three treatment groups was in excess of 99%. The timing in fibre growth (DL, GL, FGR, SS, NEDF) was 2 to 6 weeks earlier in LD goats compared to P and SD goats which were similar. When PRL was infused, via the descending iliac artery, directly to the skin, mean plasma PRL concentration was 9 (7-11) ng/ml, 110 (83-145) ng/ml and 43 (35-57) ng/ml in the venous jugular, PRL- and saline-infused iliac catheters respectively (P<0.01). The mean blood flow through the skin patch was 12±4 ml/min and 61 ml/min in goats and sheep respectively (P<0.05). There was no effect of PRL-infusion on either PA or SA. The direct infusion to the skin of PRL (from two different sources) resulted in the visible swelling of the proximal hind-leg 6.5±0.8 days after the start of the infusion. This effect was independent of species, PRL infusates established infusate sterility. In conclusion, follicle and fibre growth were unaffected by either systemic or local infusions of PRL in down-producing goats and Wiltshire sheep. However, the effects on the follicle of the infusion of PRL directly to the skin were confounded by the extreme local tissue reaction.

**Chapter 9:** The conclusions from the research programme are presented and future directions discussed.



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## ABBREVIATIONS

DGR	Down growth rate
DL	Down length
DL <sub>d</sub>	Mean down length decreased by more than 10 mm
DL <sub>min</sub>	Mean down length first reached zero
DL <sub>i</sub>	Mean down length began to increase.
DMFD	Down mean fibre diameter
DUR <sub>max-min</sub>	Number of days between the date of maximum growth and date of minimum growth.
DUR <sub>min-max</sub>	Number of days between the date of minimum growth and date of maximum growth.
FGR	Fibre growth rate
GHGR	Guard hair growth rate
GL	Guard hair length
GMFD	Guard hair mean fibre diameter
GL <sub>min</sub>	Mean guard hair length reached a minimum.
NEDF	Newly emerged down fibres
NEDF <sub>o</sub>	NEDF first appeared above the skin; NEDF score 1 or 2.
NEDF <sub>d</sub>	NEDFs disappeared; NEDF returned to 6
NEWF	Newly emerged wool fibres
PA	Primary follicle activity
PAc+brush	Primary follicle containing both actively growing and inactive fibre
PAct <sub>f</sub>	The last instance of primary follicles with both active and brush fibres
PA <sub>max</sub>	Primary follicle activity reached 100%
PRL	Prolactin
PRL100	Plasma prolactin concentration reached 100 ng/ml
PRL <sub>p</sub>	Plasma prolactin concentration reached peak concentrations
SA	Secondary follicle activity
SA <sub>s</sub>	Peak in summer secondary follicle activity
SAc+brush	Secondary follicle containing both actively growing and inactive fibre
S225	Subgroup containing 225 does
S90	Subgroup containing 90 does
S30	Subgroup containing 30 does
S15	Subgroup containing 15 does
SAct <sub>w</sub>	Second peak in proportion of secondary follicles with both active and brush fibres
SA <sub>max</sub>	Secondary follicle activity reached 100%
SA <sub>w</sub>	Secondary follicle activity increased indicating start of winter down growth
SS	Shedding score (1-5; 1=no shedding)
SS <sub>i</sub>	Shedding score first increased to 2 or greater
SS <sub>max</sub>	Shedding score reached maximum levels
SS <sub>f</sub>	Shedding score returned to 1.