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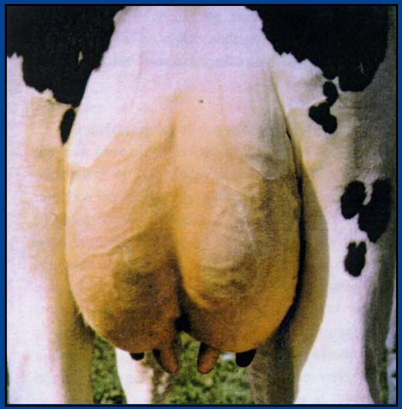
Kristen Sejrsen
August 2012

SOMETHING ABOUT MAMMARY DEVELOPMENT

Kris Sejrsen, Department of Animal Science,, Aarhus University - Foulum, Denmark



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Introduction

- Thank you for inviting me – recieved with mixed emotions
- What should I talk about?
- Recent work?
  - Milk fat composition – bioactives (IGF/IGFBP/CLA/FA)
  - Milk composition and human health
  - Mammary gene expression of lipogenic genes
  - Membrane synthesis and membrane FA composition
  - Involvement of cholestrol in regulation FA synthesis
  - Membrane transport – ABC genes (Albrecht/Bruckmeier collaboration)
- My old topic?
  - Interesting new approaches with promise
  - Interpretation of new data in conflict with
  - Things I would to work on

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Disposition

Introduction

- Capuco, Hovey, Bach, Rowan

Phases of mammary development – overview

Fetal period

- Intro – Kenyon et al.
- Milk yield
- Mammary development

Preweaning

- Intro
- Milk yield /Foldager/Bar-Peled/Bach 2012
- Mammary development
  - Sejrsen/Brown/Daniels
- Mechanism of action – proteomics/RNA

Pubertal

- Intro –
  - Sinha & Tucker/
  - OVX – Purup
  - Ellis – Myoepithelial cells
- Milk yield
  - Curves – Sejrsen 2000, Lammers& Heinrich
  - Variable results – why?
  - Genetic vs. nutritional
  - BW correction – Amhurg/Lønne
- Mammary development
  - Results – Swanson/Sejrsen/Meyers
  - Age vs. BW
  - Uncoupling from reproductive organs
- Mechanism of action
  - Adipose tissue/fat pad (Faulkin/Swanson/Hovey)
  - Endocrine GH/IGF
  - Local – leptin/PNF-alfa/TGF-Beta/IGF-BP3
  - Stem cells
  - Growth zones

Postpubertal

- Intro
  - Sinha and Tucker/Sejrsen 1982/Myers
  - Sejrsen & Foldager/Myers/Sejrsen. Enright/Petitclerc
- Milk yield
  - Sejrsen & Foldager
- Mammary
  - Sejrsen et al./ Petitclerc

Pregnancy

- Intro
- Milk Yield - Lønne repeat/ Petitclerc
- Mammary – Capuco/Sørensen

Lactation

- Intro
  - Importance of catation curve
  - Sørensen/CapucoAnnen et al.
  - Sørensen/Capuco
  - Early lactation important/ Dahl/Wall et al
- Milk yield
  - Push/pull – theory – Andersen et al.
- Mammary
  - Capuco/Nørgaard/Desuage

Concluding remarks

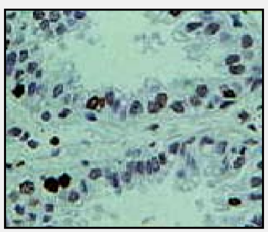
Stages of postnatal mammary development

Galactopoesis
Maintenace of lactation

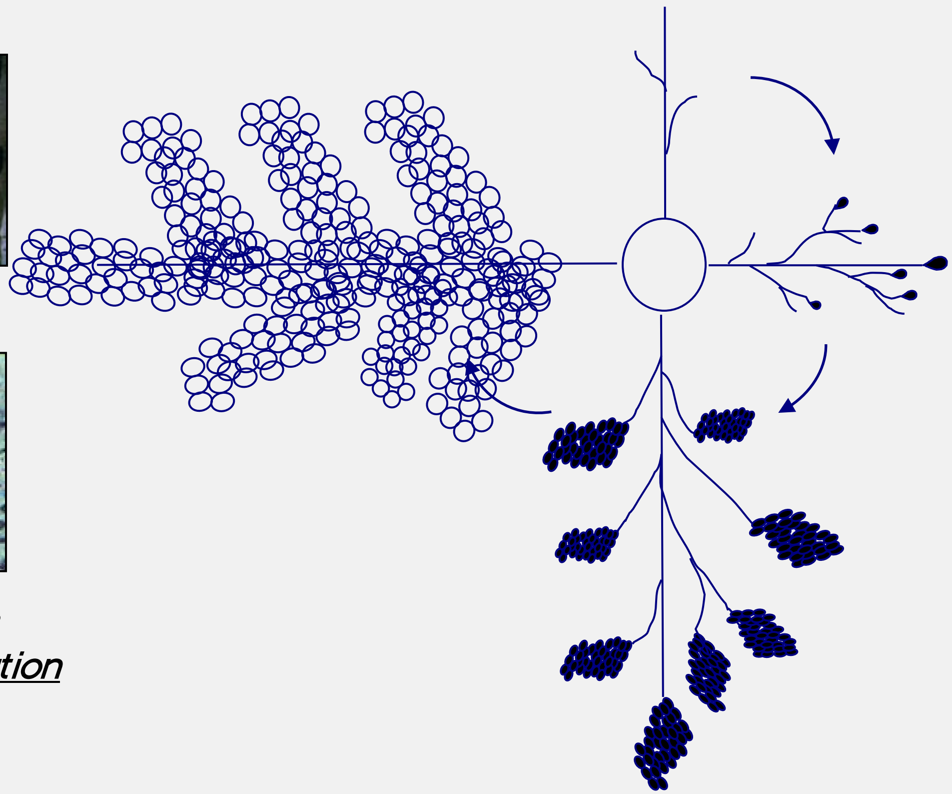
Mammaogenesis
Pubertal period

Rudimentary
mammary ducts

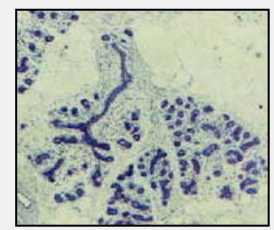
Differentiation



Lactogenesis
Initiation of lactation



Ducts



Mammogenesis
Pregnancy

Alveoli
Ducts
Stroma

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FETAL PERIOD

Interesting new data from New Zealand

Nourishing and managing the dam and postnatal calf for optimal lactation, reproduction, and immunity. Van Linden et al, 2009.

Trait	Treatment[1]				*P*-value	
	A	M	H	L	Nutrition effect	Size effect
Fetal wt, g	1,228 ± 41.3	1,129 ± 69.2	1,213 ± 47.1	1,144 ± 65.4	0.24	0.40
Fetal mammary gland wt, g	**3.98 ± 0.12**	**4.53 ± 0.20**	**4.16 ± 0.13**	**4.35 ± 0.19**	**0.03**	**0.43**
Total duct area, $\mu m^2 \times 10^2$	3,714 ± 320.2	3,571 ± 517.6	4,866 ± 352.7	2,419 ± 496.0	0.82	0.0008
Number of ducts	49.7 ± 3.39	48.0 ± 5.48	51.9 ± 3.73	45.8 ± 5.25	0.79	0.36
Number of ducts with lumen	42.2 ± 3.09	42.3 ± 4.99	44.6 ± 3.40	40.0 ± 4.78	0.99	0.44

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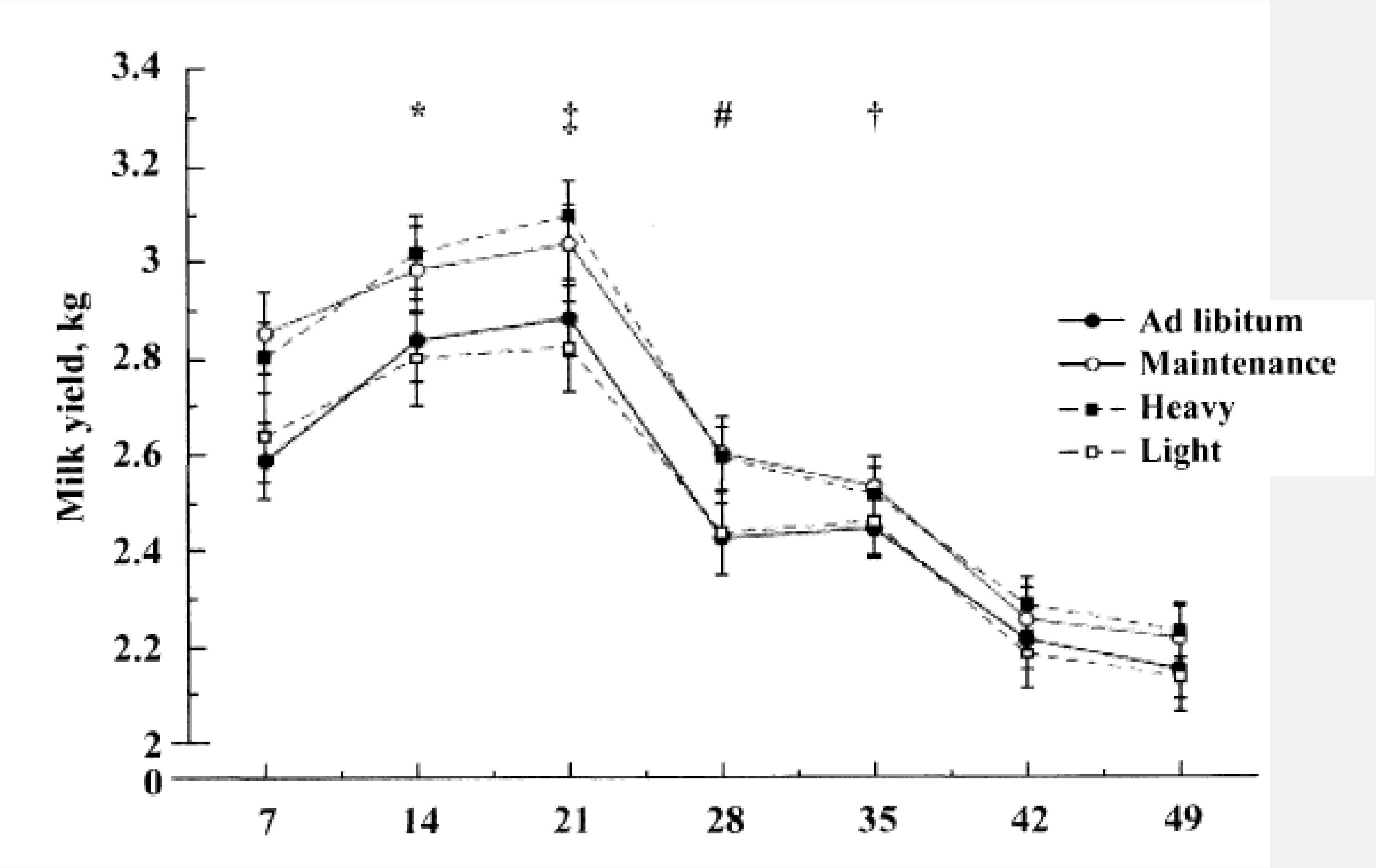

PREWEANING PERIOD

Focus of a lot of research the last 10 years

- Recent review by Bach, 2012.
- Lenght of preweaning period?
- Increased yield due to increased mammary growth?

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RUMINANT NUTRITION SYMPOSIUM: OPTIMIZING PERFORMANCE OF THE OFFSPRING: Nourishing and managing the dam and postnatal calf for optimal lactation, reproduction, and immunity

A. Bach

J ANIM SCI 2012, 90:1835-1845.

Study	Treatment[1]	n	Change in ADG, g/d	Change in BW at calving, kg	*P*-value[2]	Change in milk yield, %	*P*-value[3]
Bar-Peled et al., 1997	Suckling the dam 3X vs. MR 1X (360 g/d)	14 to 15	100	37	NS	**4.0**	<0.10
Shamay et al., 2005	WM 2X (ad libitum) vs. MR 1X (450 g/d)	10	290	21	NS	**4.5**[a]	<0.05
Moallem et al., 2010	WM 2X (1.2 kg/d) vs. MR 2X (1.1 kg/d)	8 to 9	74	38	0.05[a]	**13**[a]	<0.05
Davis Rincker et al., 2011	MR 2X (1,030 kg/d) vs. MR 2X (600 g/d)	30	200	−9	NS	**4.1**	<0.10[b]
Morrison et al., 2009	MR 2X (1,200 g/d) vs. MR 2X (600 g/d)	40	160	2	NS	**−1.3**	NS
Raeth-Knight et al., 2009	MR 2X (990 g/d) vs. MR 2X (475 g/d)	22	240	—	—	**5.2**	NS
Terré et al., 2009	MR 2X (410 g/d) vs. MR 2X (900 g/d)	14	100	—	—	**5.9**	NS

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Residual milk (kg) for first, second, and third lactation as well as cumulative milk from first through third lactation by ADG before weaning for calves in the **Cornell herd**. Soberon et al. 2012.[1]

Lactation	No. of animals	Predicted difference in milk per kilogram of preweaning ADG	*P*-value
First	1,244	849.63	<0.01
Second	826	888.08	<0.01
Third	450	48.32	0.91
First through third	450	2,279.53	0.01

Residual milk (kg) for first, second, and third lactation as well as cumulative milk from first through third lactation by ADG before weaning for the **commercial herd**. Soberon et al. 2012.

Lactation	No. of animals	Predicted difference in milk per kilogram of preweaning ADG	*P*-value
First	623	1,113.97	0.03
Second	484	−526.44	0.49
Third	271	1,293.47	0.18
First through third	271	1,286.18	0.51

Influence of postnatal growth on yield, (Foldager et al.,1997)

	4.2 kg milk (0-6 weeks)	Milk ad lib. (0-6 weeks)	Suckling (0-12 weeks)
ADG (g)			
- 0-6 weeks	660	960	963
- 6-12 weeks	635	523	1036
BW (kg)			
- at 6 weeks	73	85	85
- at 12 weeks	99	107	130
Milk (kg/d)	25.3[a]	26.9[ax]	24.4[b]

a vs ax; $p < 0.10$
ax vs b, $p < 0.05$

Milk yield in relation postnatal feeding level, (Bar-Peled et al., 1997)

	Control	Suckling (0-6 weeks)
ADG (g) 0-6 weeks	560	850**
ADG (g) 6-12 weeks	860	350*
Milk (kg) 300d	9171	9624[a]

a: $p < 0.10$

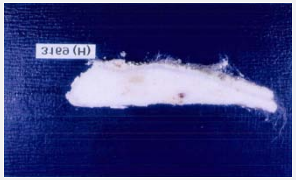
The results by Foldager et al. and Bar-Peled suggest that the positive effect ends at 6 weeks of age

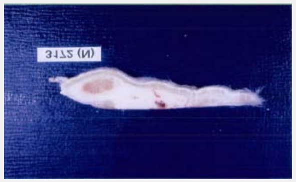
Influence of postnatal feeding level on mammary growth (Sejrsen et al., 1998)

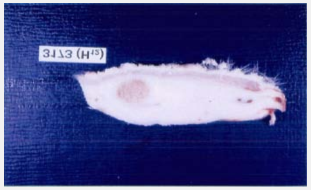
Milk 0-6 weeks, kg		4.5 kg	Ad lib.
Feeding level – 6 wks – 250 kg		M	M
ADG (g) 5 – 42 dage		**632[a]**	**883[b]**
ADG (g) 42 dg – 250 kg		**758[a]**	**713[a]**
Mammary parenchyma			
- At 6 weeks	- BW,kg	-	83
	- Par, g/100kg	-	4.6
- At 12 weeks	- BW,kg	93	110[a]
	- Par, g/100kg	20,5	21,8
- At 250 kg	- Age, d	270	257
	- Par, g/100kg	347[a]	440[a]

Daniels et al. 2009 found similar results

Brown et al. 2005 observed increased parenchymal growth from 2-8 weeks







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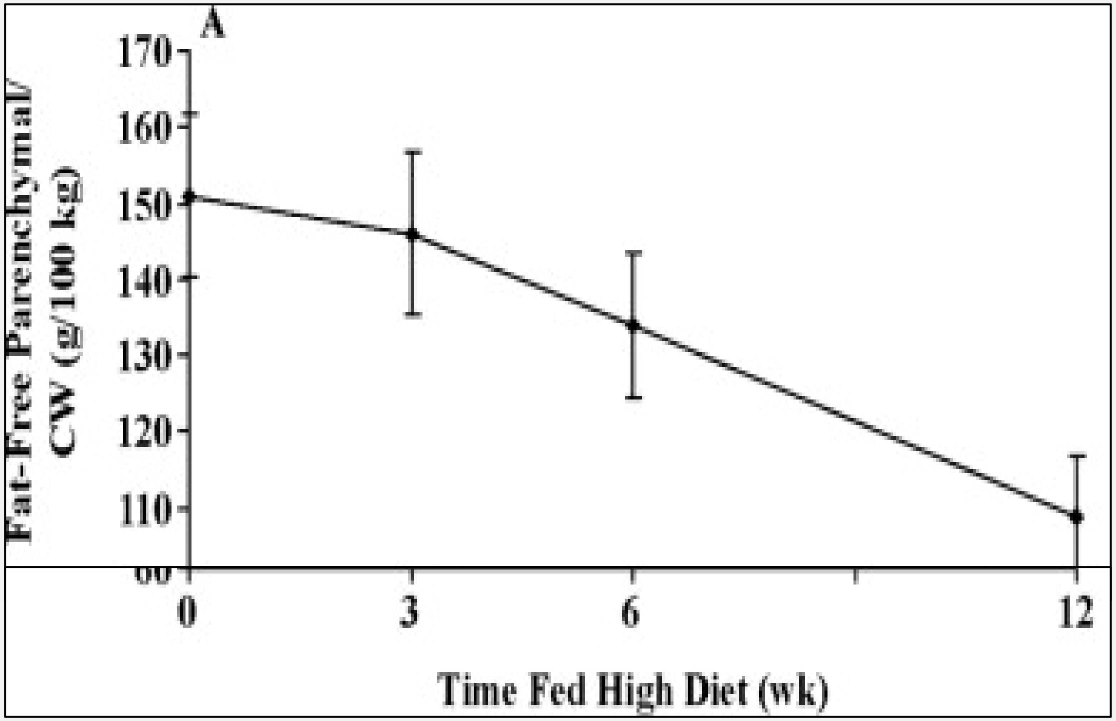
Effects of Feeding Prepubertal Heifers a High-Energy Diet for Three, Six, or Twelve Weeks on Mammary Growth and Composition



L. E. Davis Rincker,*1 M. S. Weber Nielsen,* L. T. Chapin,* J. S. Liesman,* K. M. Daniels,† R. M. Akers,† and M. J. VandeHaar

PREPUBERTAL PERIOD AND MILK YIELD

Conclusions drawn from newer data ignores old results

- Is this correct or not?
- I don’t think so and
- I will try to explain why

Swanson et al 1960

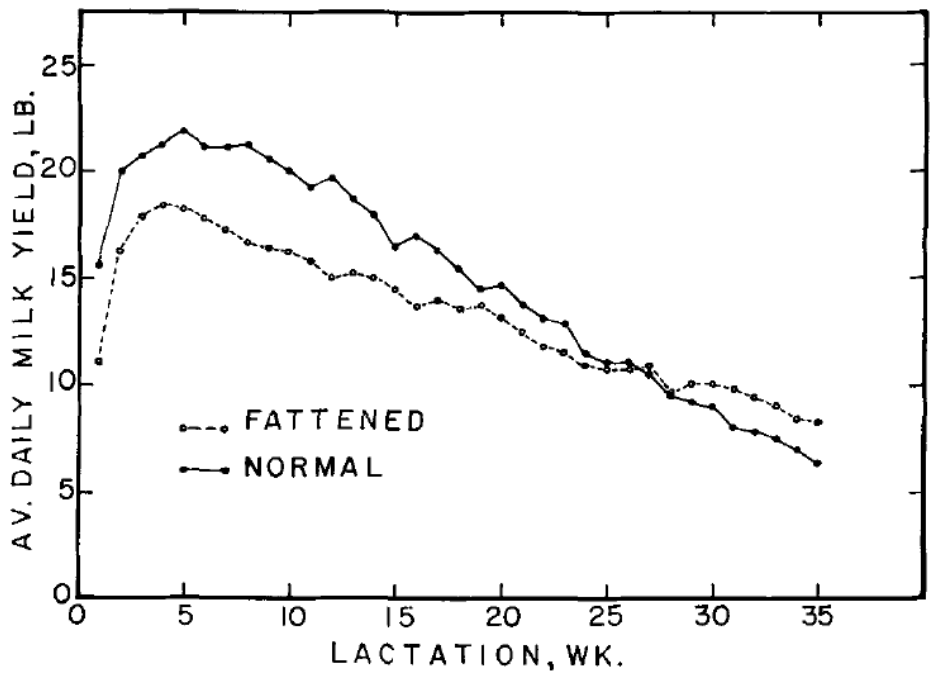
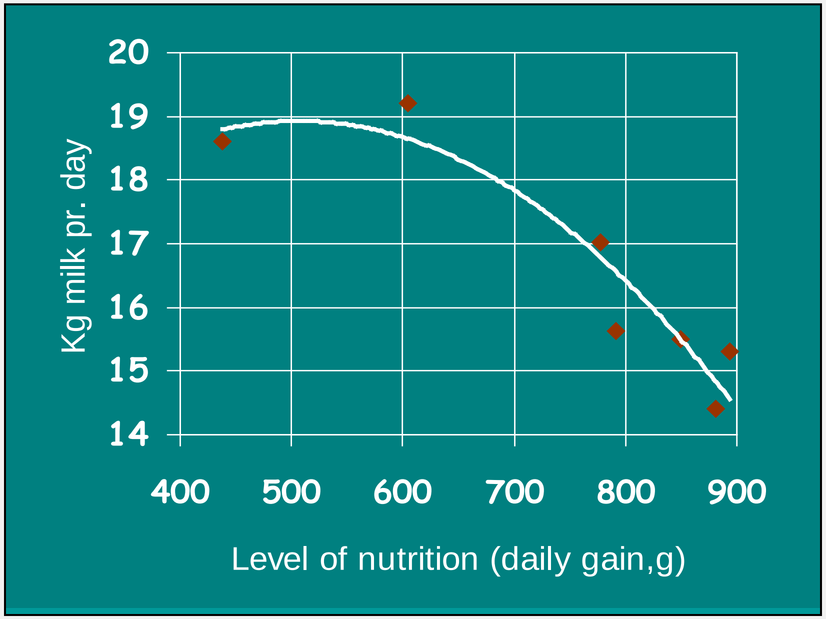


FIG. 4. Average lactation curves of seven pairs of identical twins, comparing fattening vs. normal feeding prior to first calving.

Feeding level and prepubertal mammary development



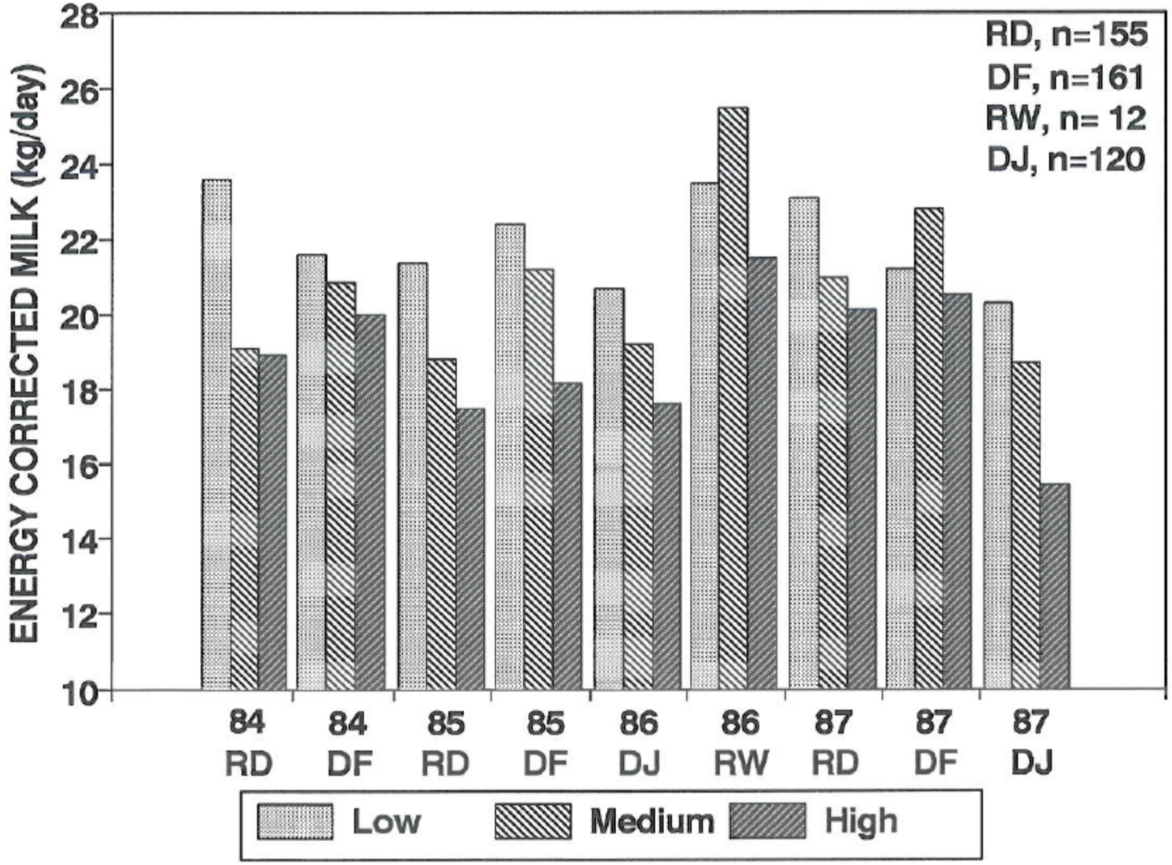
Moderate feeding level

(ADG 700 g/d)

High feeding level

(ADG 1150 g/d)

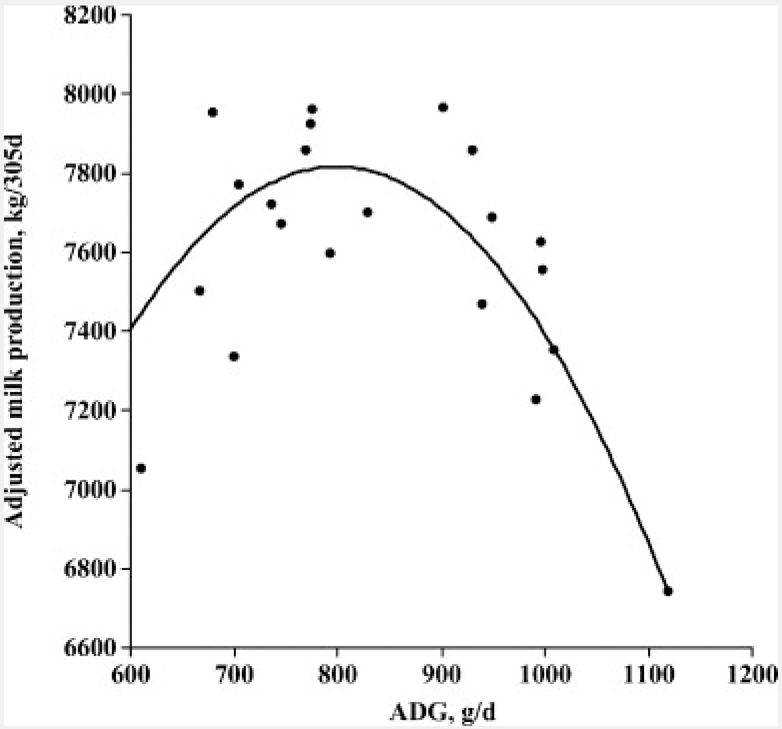
Influence of feeding level on milk yield

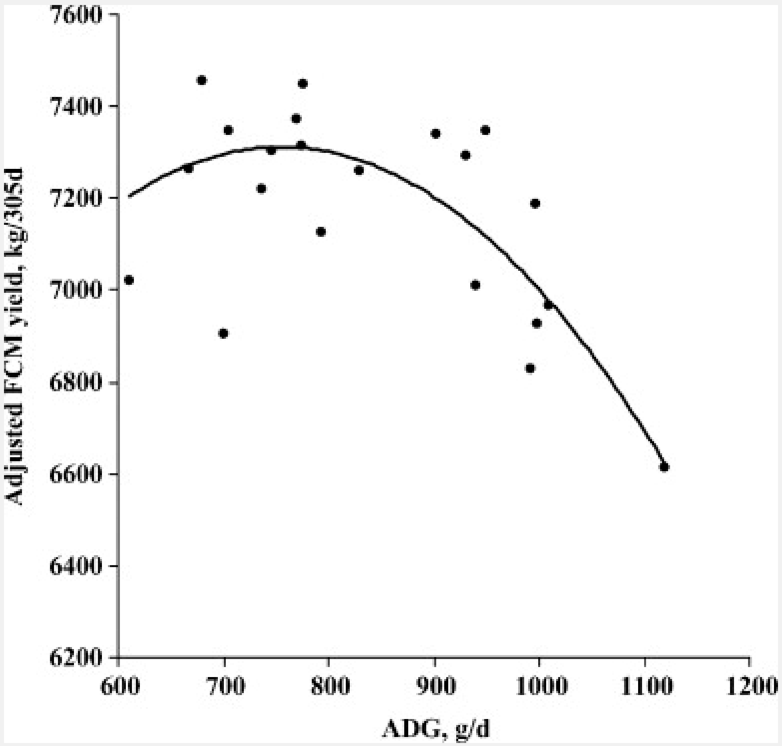


Hohenboken, Foldager, Jensen et al., 1994

Data from 8 individual studies

G. I. Zanton and A. J. Heinrichs, 2005,J. Dairy Sci. 88:3860–3867





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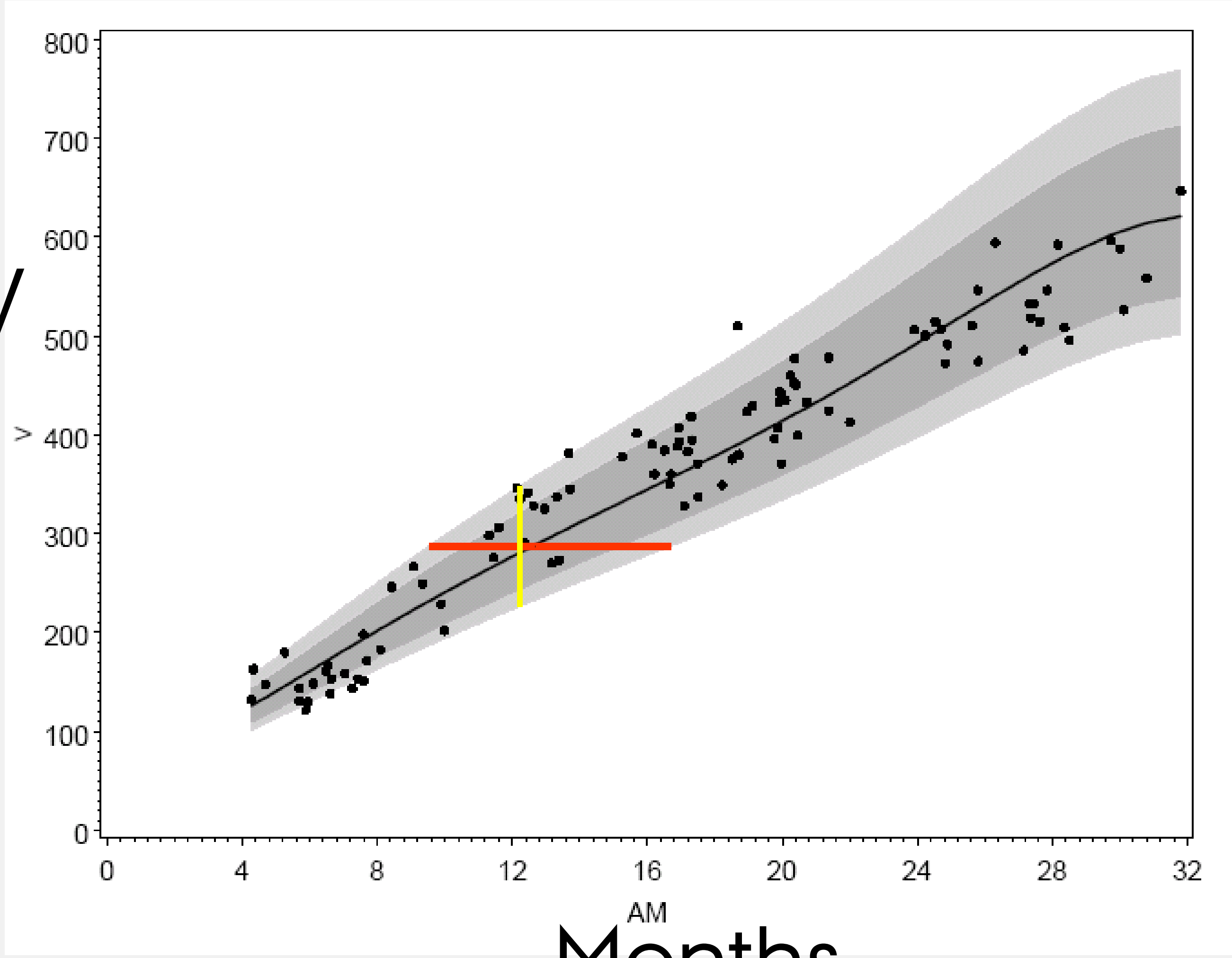
Question:

- Why does many recent exp. not come to the same conclusion?
-

- Possible explanations?
    - Feeding level and ADG is not the same
    - Faulty correction for genetic based variation in BW at calving
    - Large variation in AGD within group at same feeding level
    - Feeding levels does not include optimum feeding level
    - Treatment period include preweaning period
        - Positive pre weaning effect offset negative prepubertal effect

BW of individual heifers compared to plo

BW



Months

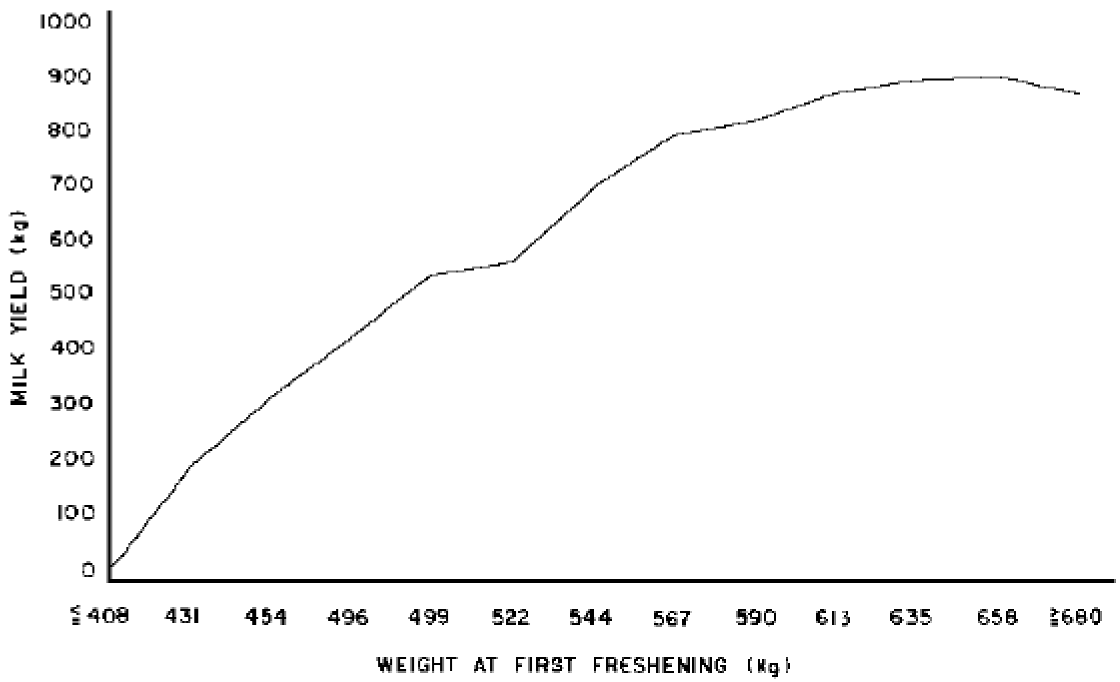


Figure 1. Relationship between first lactation milk yield and body weight.

Keown & Everett, 1986

Differences in test-day model residual milk (kg) for first, second, and third lactation as well as cumulative milk from first through third lactation by ADG from weaning to breeding for the commercial herd. Soberon et al. 2012.

Lactation	Predicted difference in milk per kilogram of ADG from weaning to breeding[1]	*P*-value
First	1,168.48	0.10
Second	2,719.87	0.01
Third	2,874.88	0.05
First through third	8,199.80	<0.01

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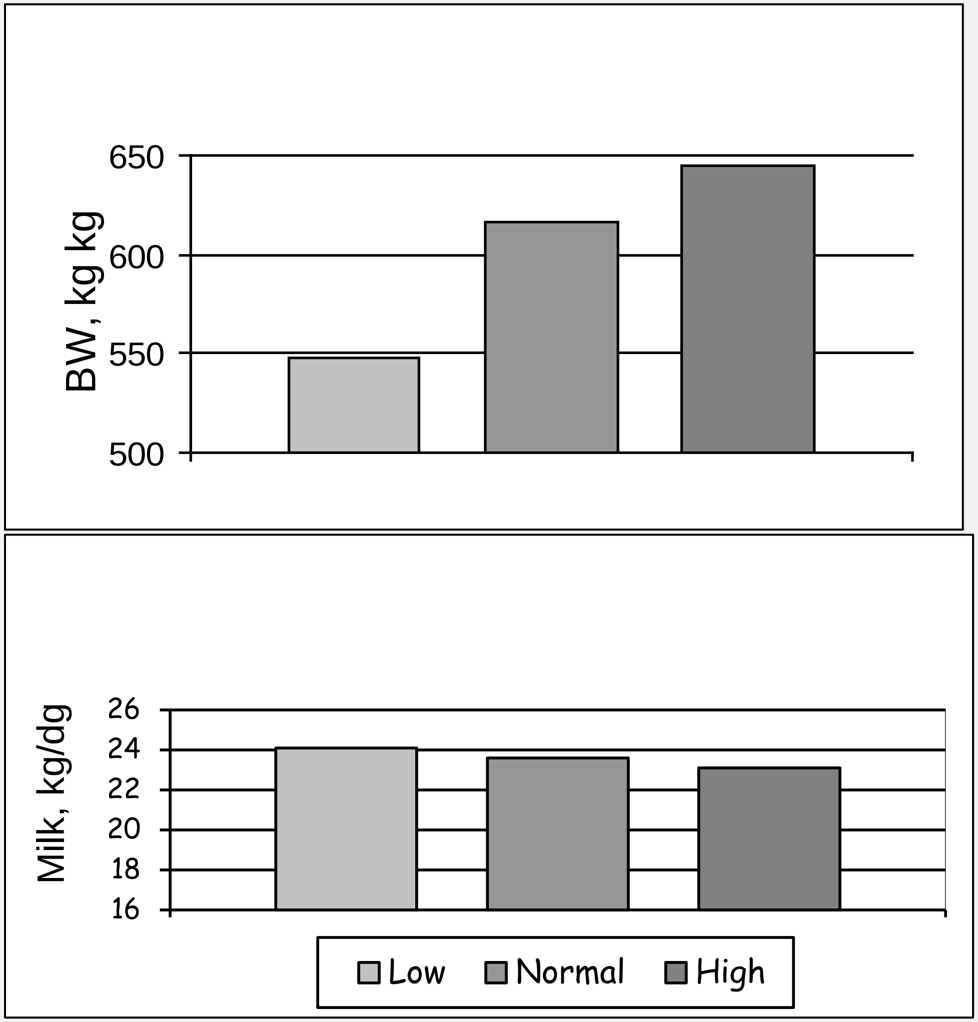
Influence of feeding level during pregnancy on BW at calving and milk yield



Ingvartsen et al., 1995

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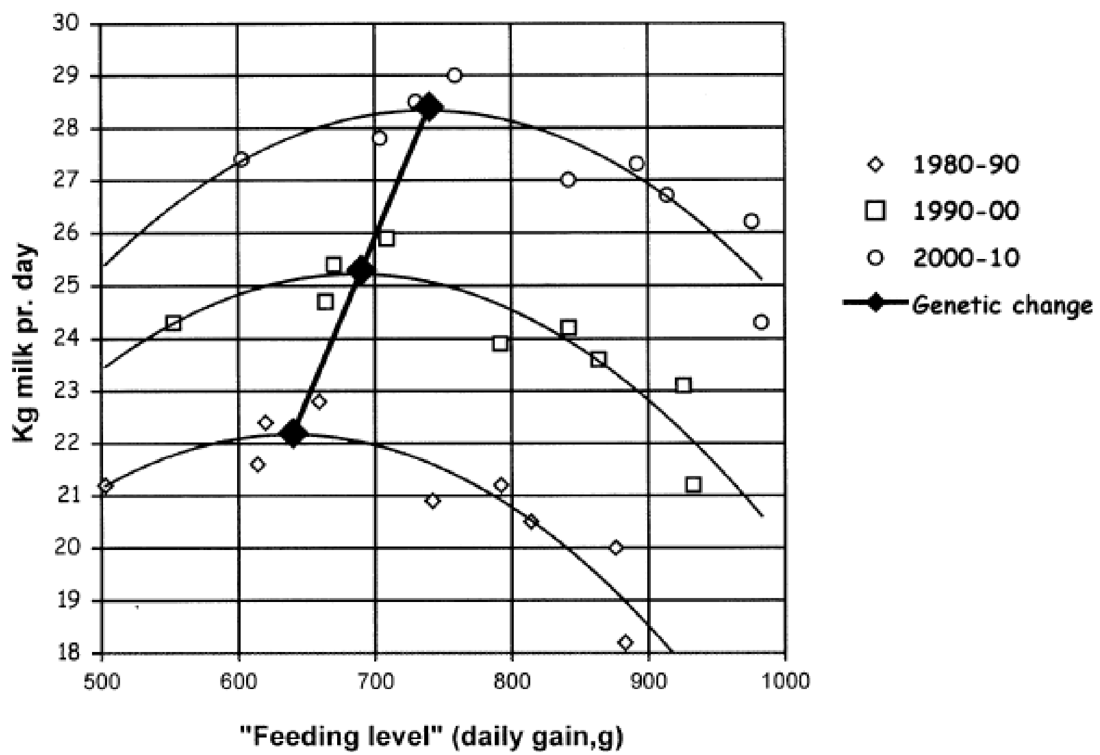
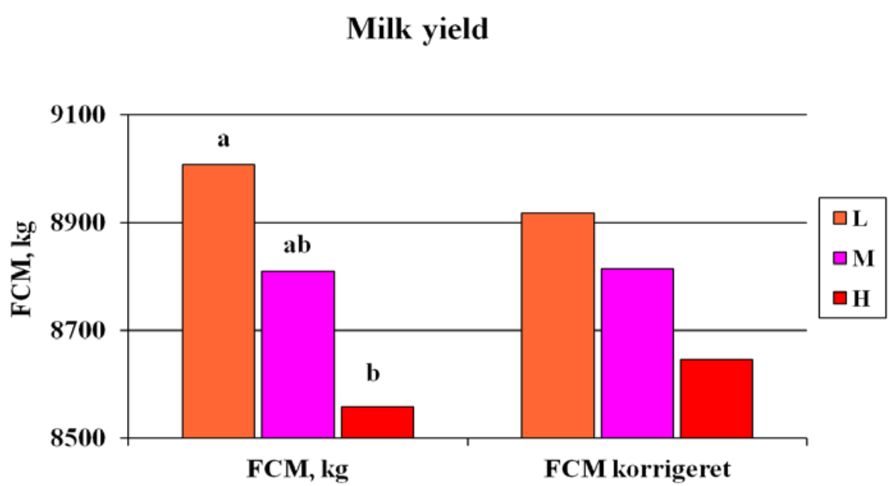


Fig. 6. Illustration of the expected change in optimal daily gain with the increased genetic potential in milk yield from the 1980's to this decade. The relationship between feeding level and milk yield is unchanged and the higher optimal daily gain is achieved at the same feeding level.

Feeding level between 90 and 320 kg BW on milk yield in 1st lactation, Van Amburgh et al. 1998

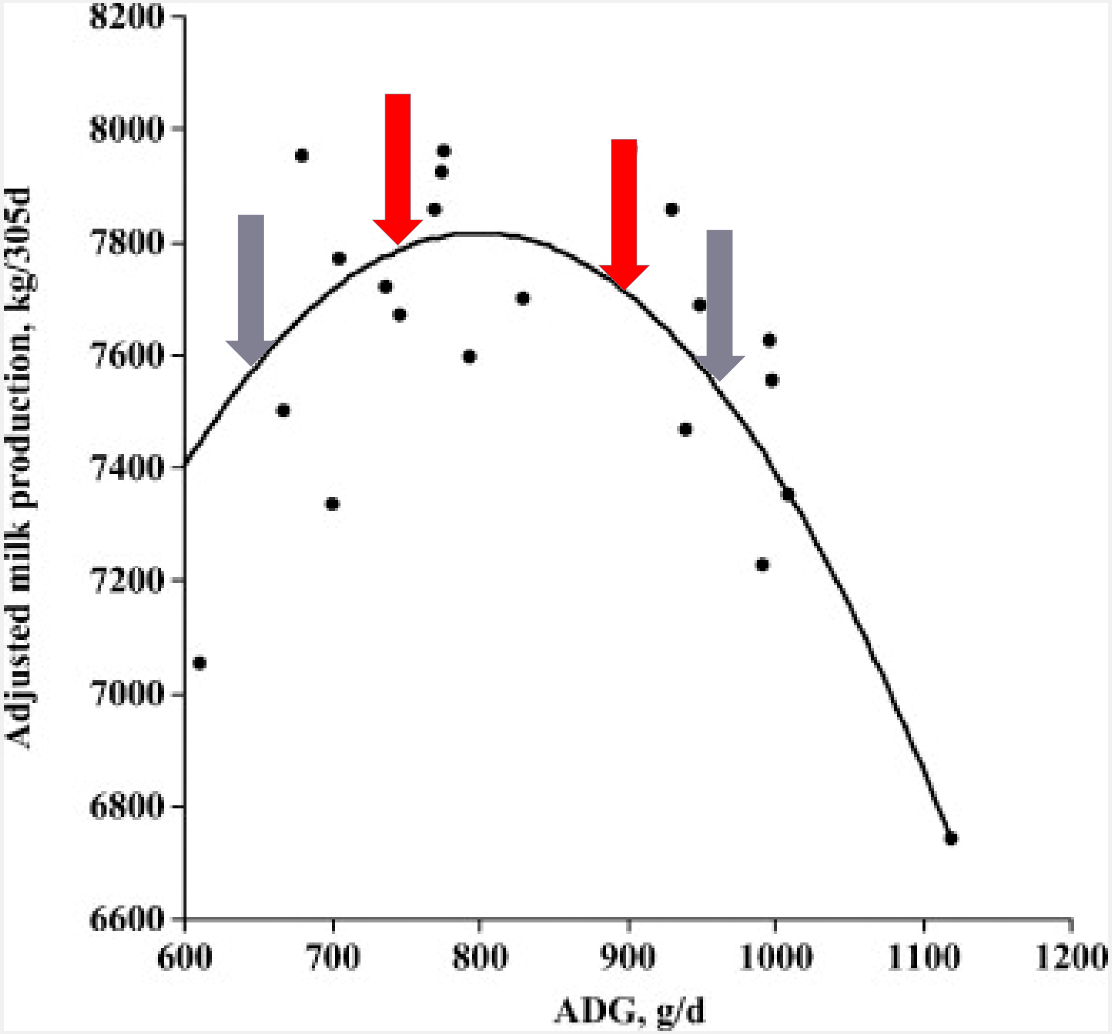


ADG	0.6	0.8	1.0
BW at calving	550	529	520

- Effect disappeared by correction for BW
- Treatment started at birth
- So positive effect of preweaning treatment affect the result

Data from 8 individual studies

G. I. Zanton and A. J. Heinrichs, 2005,J. Dairy Sci. 88:3860–3867



PREPUBERTAL MAMMARY DEVELOPMENT

- Regulation of onset and end of start of allometric growth

- Effect of nutrition –
    - expressed on BW basis
    - on age basis?

- Mechanisms of action

- Interesting areas of research

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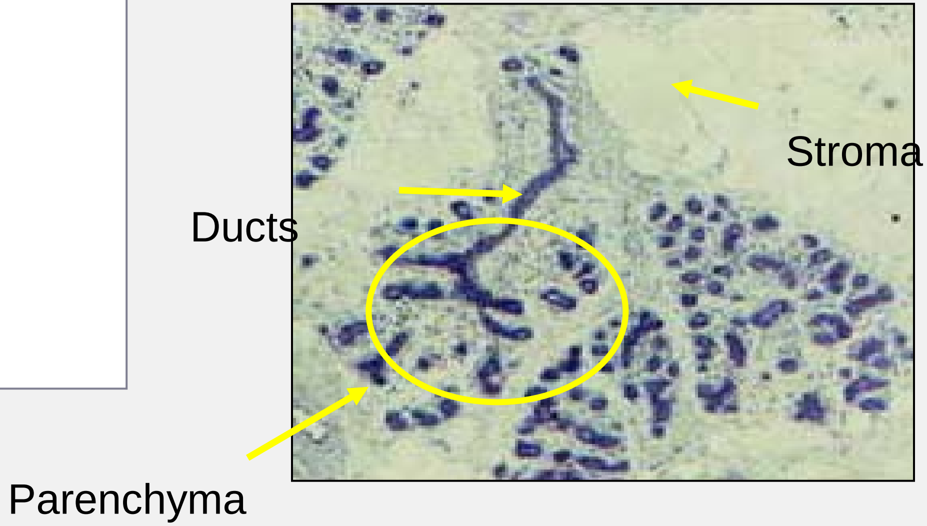
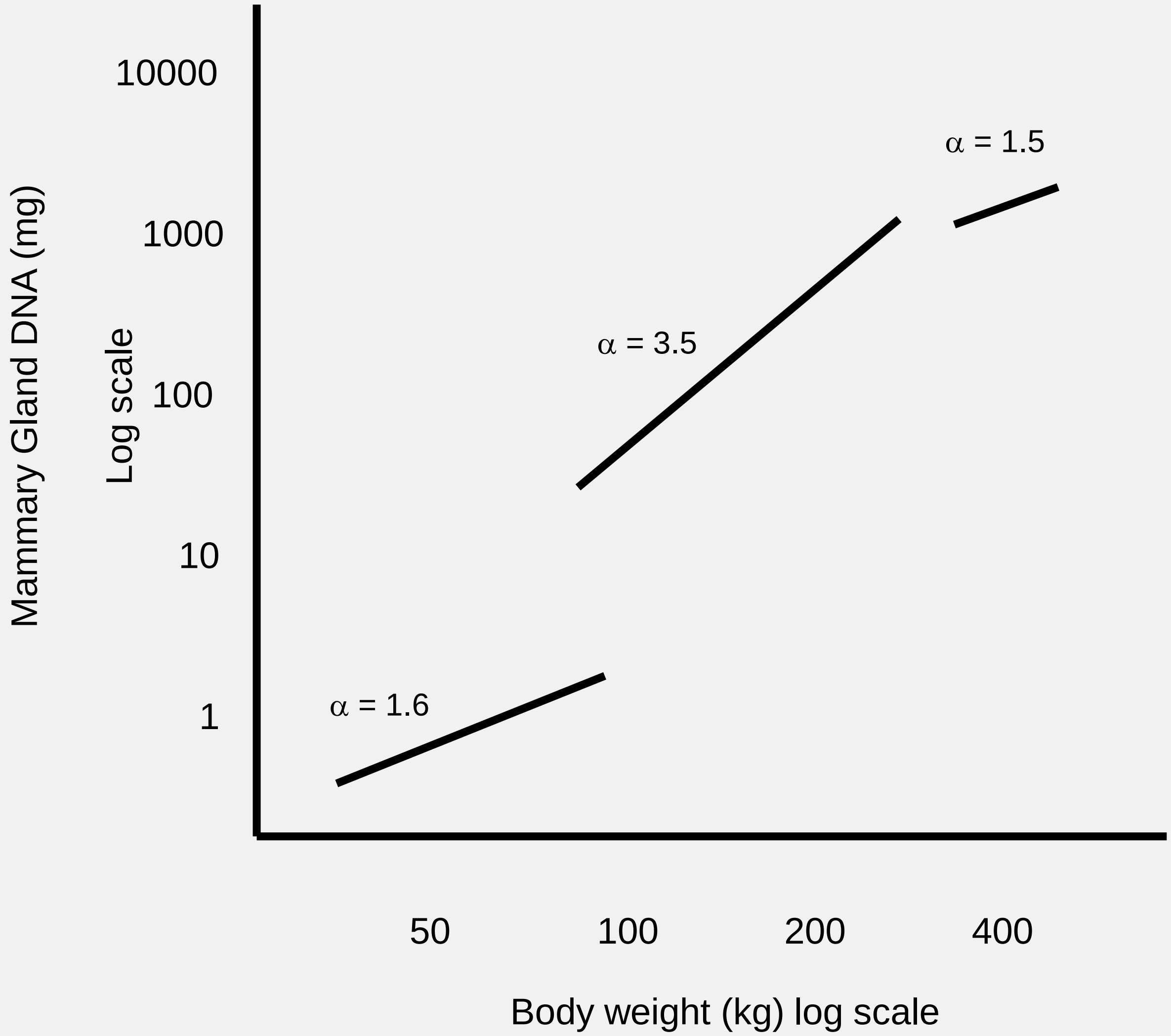
Pubertal mammary development

- Allometric growth - from 3-11 mdr
- 4 individual glands
- Growth of mammary ducts
  - *Stops before reaching the periphery*
- Parenchyma
  - *Ducts + supportive tissue*
- Stroma
  - *Connective and adipose tissue*
- No alveoli
- Critical period in relation to feeding level
- Weight 2-3 kg



Pubertal and post pubertal mammary development



Sinha & Tucker, 1969

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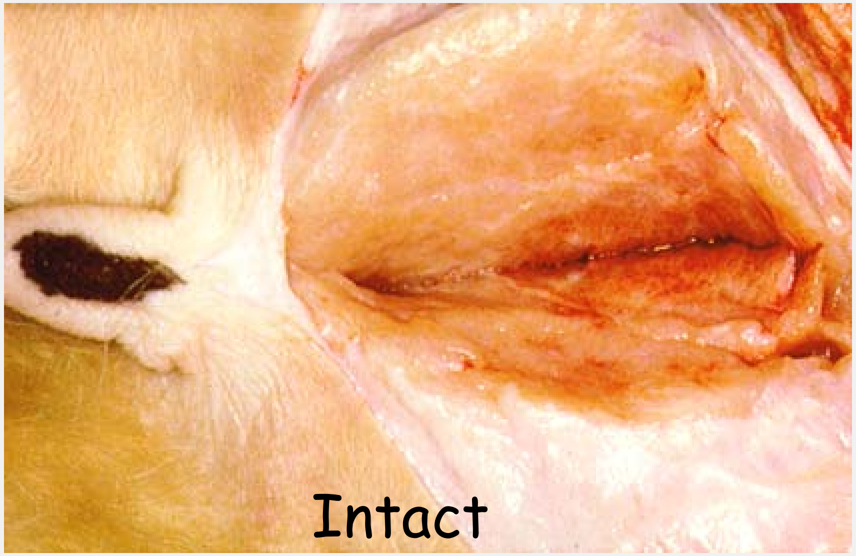

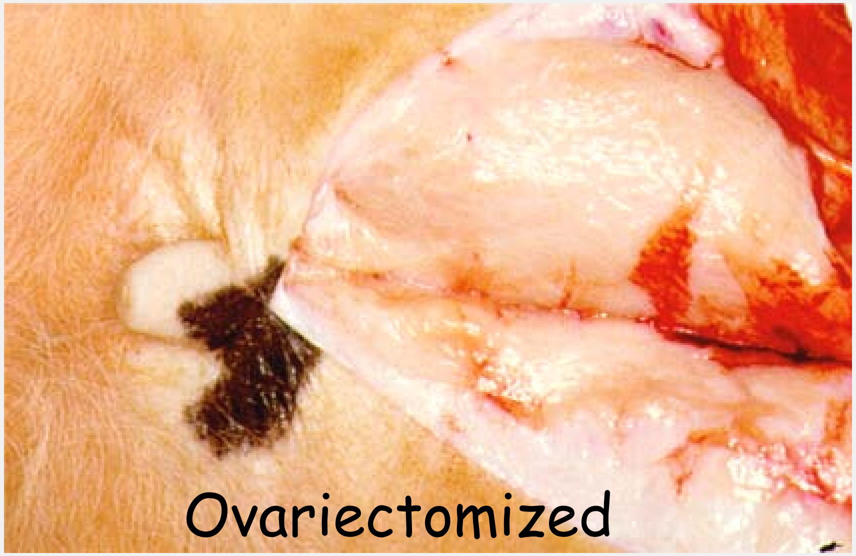

The role of oestrogen established in rodents - Lyons et al. 1958
Estrogen also required in heifers – at very low concentration
Ovariectomy completely stops mammary development
- Purup et al., 1993. J. Endocrinology
Oestrogen replacement restores mammary development in heifers – Wallace
Extensively studied by Akers and co-workers including Steve Ellis
Steve has studied the potential role of myo-epithelial cells

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TRIENNIAL LACTATION SYMPOSIUM: Bovine mammary epithelial cell lineages and parenchymal development[1,2]

S. Ellis,*[3] R. M. Akers,† A. V. Capuco,‡ and S. Safayi*

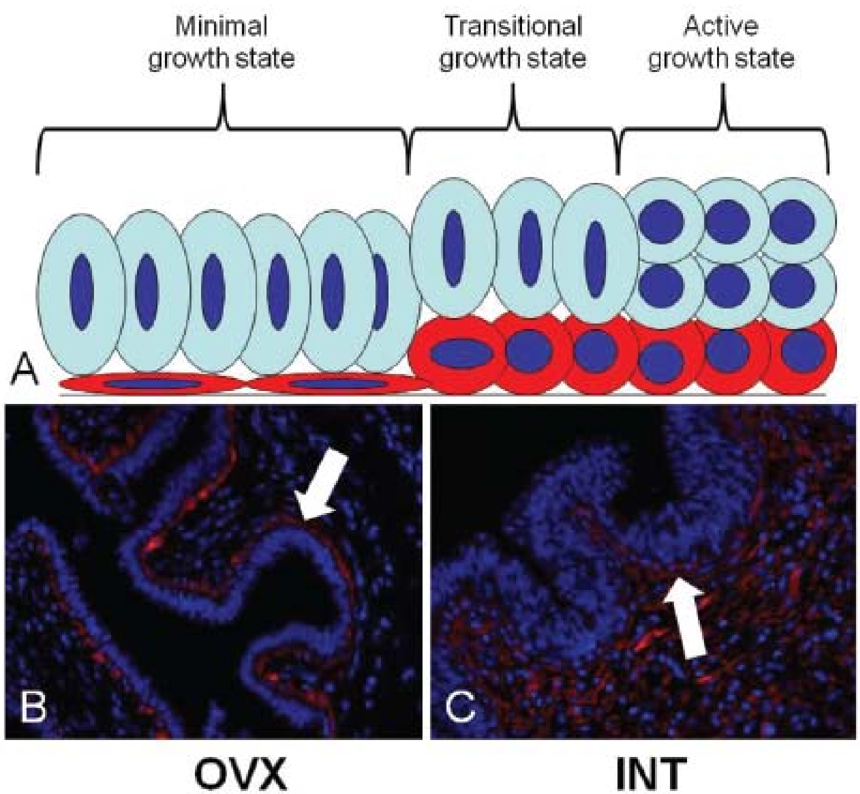

Figure 1. Prepubertal bovine parenchymal histology. Panel A depicts the general architecture of prepubertal bovine mammary epithelia during periods of active, transitional, and minimal growth. Panels B and C provide further illustration of architectural changes observed after ovariectomy (OVX, panel B), compared with age-matched intact animals (INT, panel C). Immunofluorescent staining for calponin (in red) was used to highlight the myoepithelial cells (arrows). The sections are counter-stained with PoPo-1 (blue) to highlight nuclear regions and make epithelial stratification more plainly visible.

end bud. We speculate that parenchymal development in ovariectomized heifers is inhibited by the development of a continuous myoepithelial cell layer, coupled with the attendant changes in epithelial stratification and physical separation of progenitor cells from the basement membrane.

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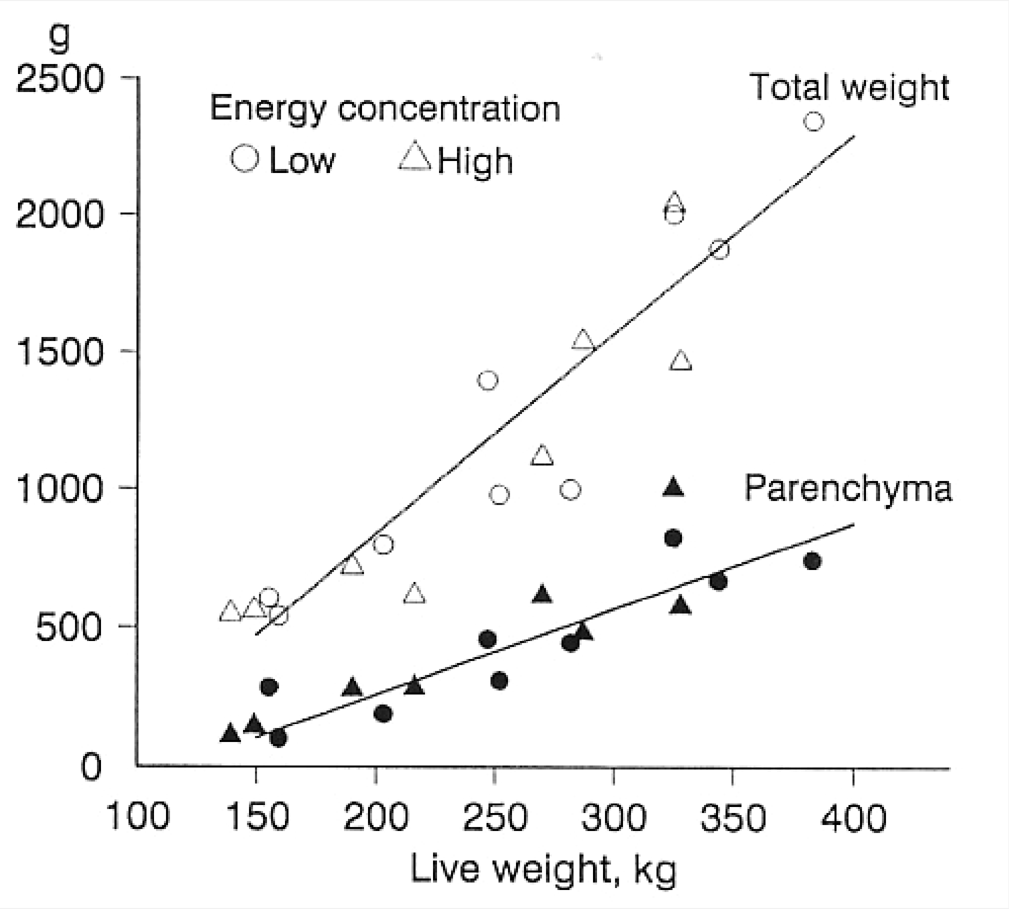
Effect of energy concentration before puberty on mammary development. (Sejrsen & Foldager, 1992)

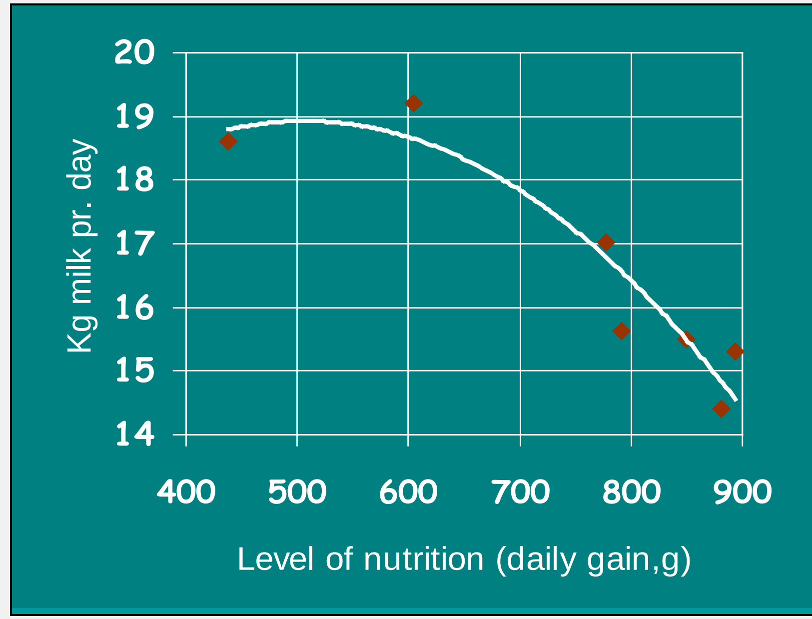


Influence of anti-GnRH immunization of beef heifers Sejrsen et al 1994.

	CONTROL	Anti-GnRH	p<
Number of animals	**10**	**11**	
Antibody titre	**1.1**	**52.2**	**0.001**
LH, ng/ml	1.51	0.98	0.001
FSH, ng/ml	24.0	14.9	0.001
IGF-I, ng/ml	107	90	0.047
Days to first cycle	**160**	**-**	
Live weight, puberty, kg	**320**	**-**	
Total gland, g.	**2956**	**3626**	**0.028**
Stroma, g.	**2334**	**3007**	**0.035**
Parenchyma, g.	**622**	**619**	**0.980**

- Asynchronous secretion of E and P may cause the return to isometric growth (Tucker)
- Not supported by these data.
- Explanation?

Feeding level and prepubertal mammary development



Moderate feeding level

(ADG 700 g/d)

High feeding level

(ADG 1150 g/d)

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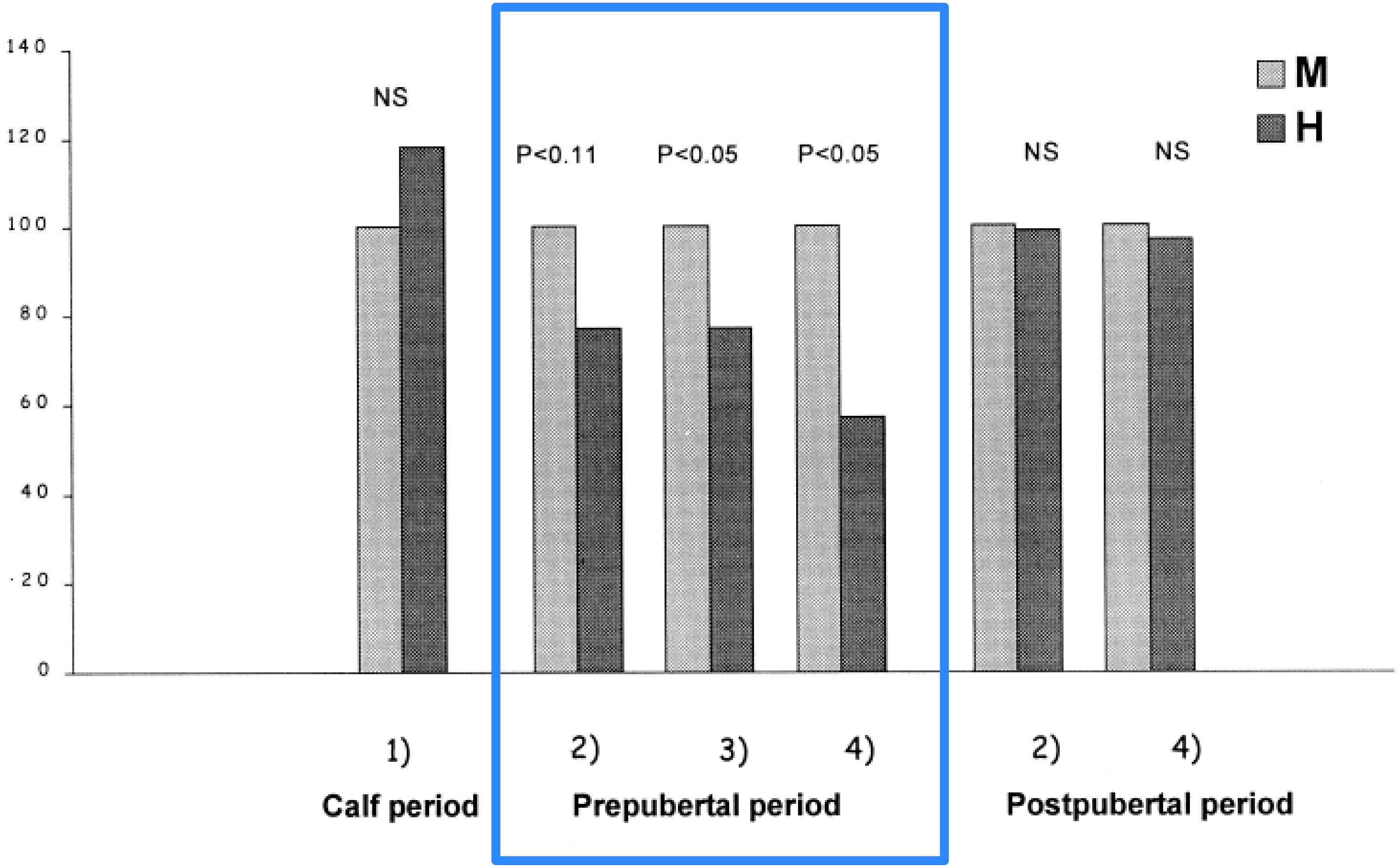
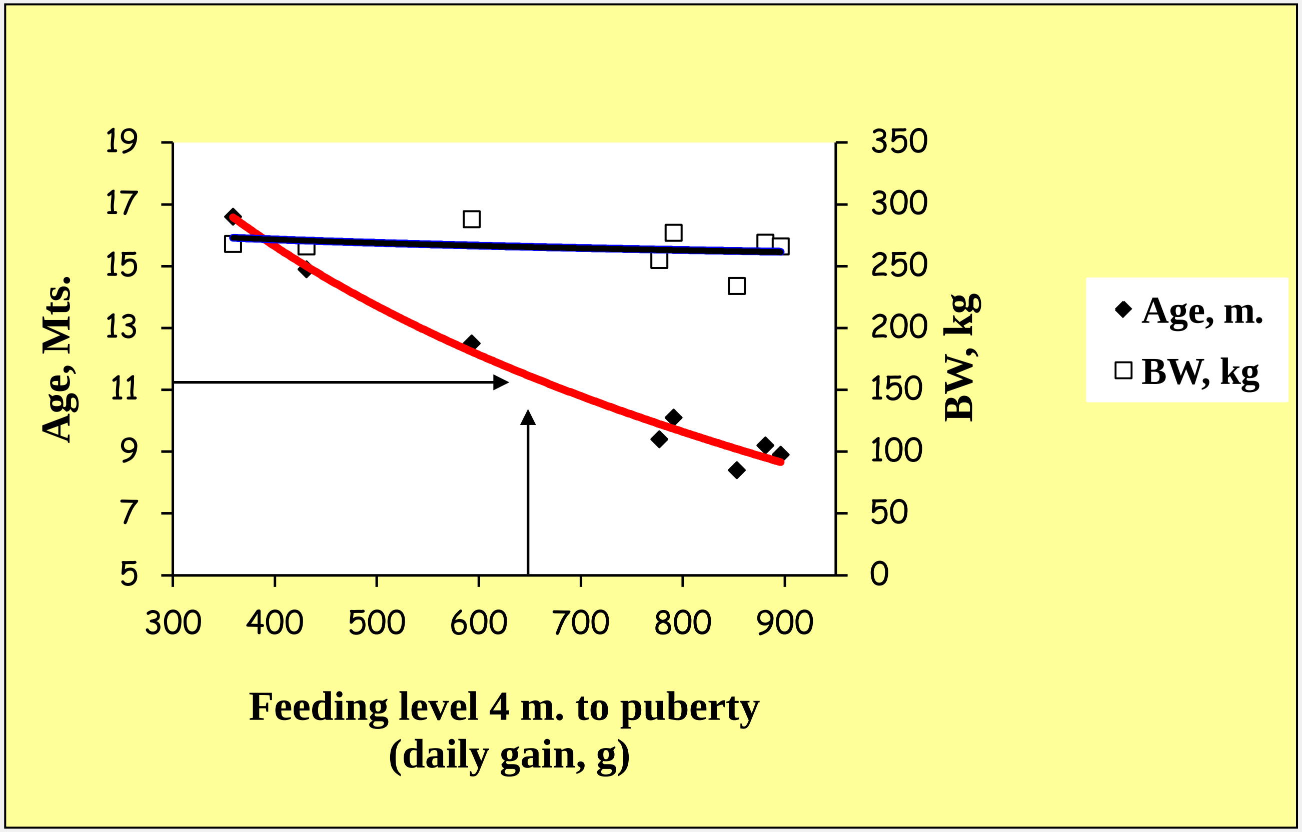
Influence of nutrition



Ref.: 1) Sejrsen et al [16] 2) Sejrsen et al [7] 3) Mäntysaari et al [15] 4) Foldager & Sejrsen [9]

Fig. 1. Mammary growth in heifers fed moderate (M) or high (H) feeding level in different stages of development.

Influence of feeding level on age and BW at first oestrus



Foldager& Sejrsen, 1991, Sejrsen et al 1994

Prepubertal uncoupling of development of mammary parenchyma and and reproductive organ

Sejrsen et al. 1982 and Spicer et al 1984

	Pubertal		
	M	H	Relative
Parenchyma, g	642	495	**77**
Uterus	165	180	**109**
Ovaries	11.9	13.0	**109**

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M. J. Meyer,*[1] A. V. Capuco,† D. A. Ross,* L. M. Lintault,* and M. E. Van Amburgh*[2]

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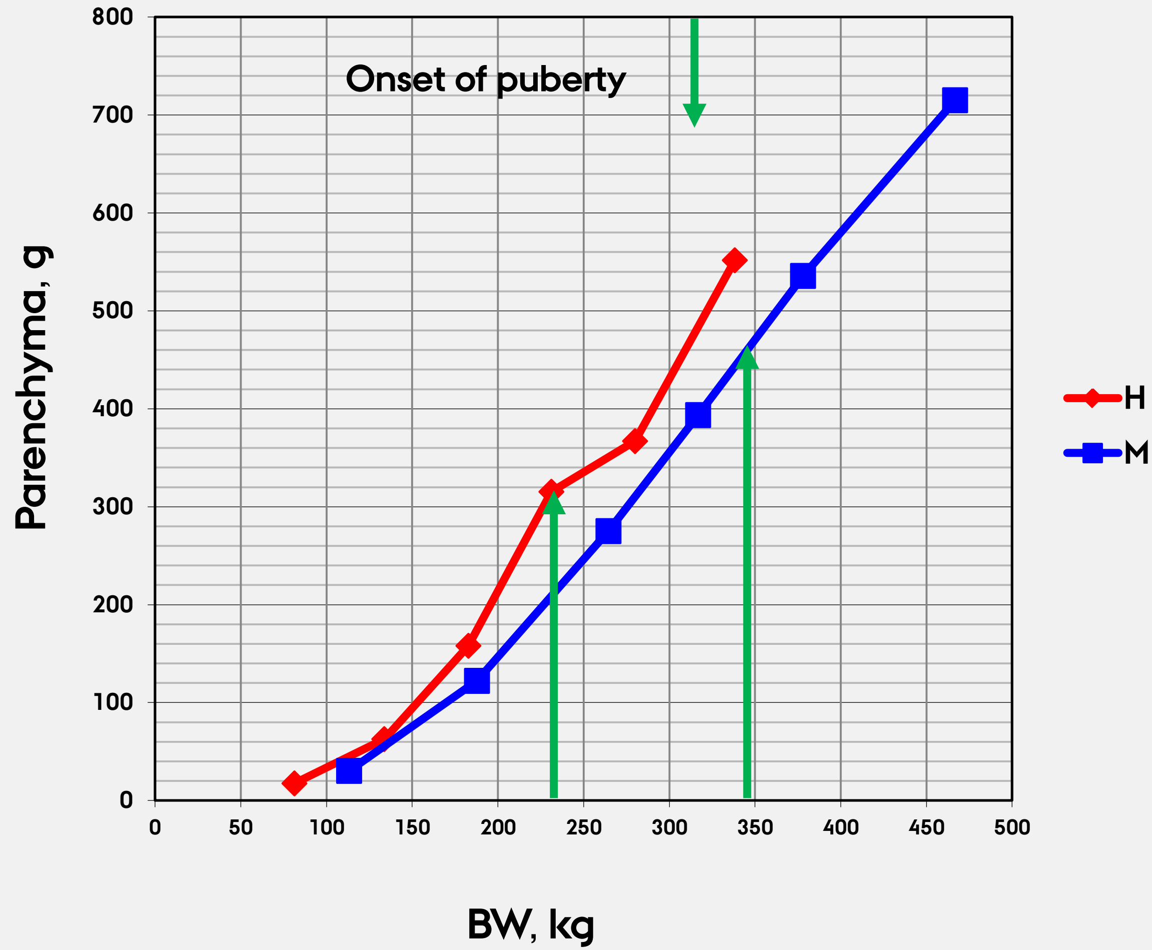

M. J. Meyer,*[1] A. V. Capuco,† D. A. Ross,* L. M. Lintault,* and M. E. Van Amburgh*[2]

Figure 3

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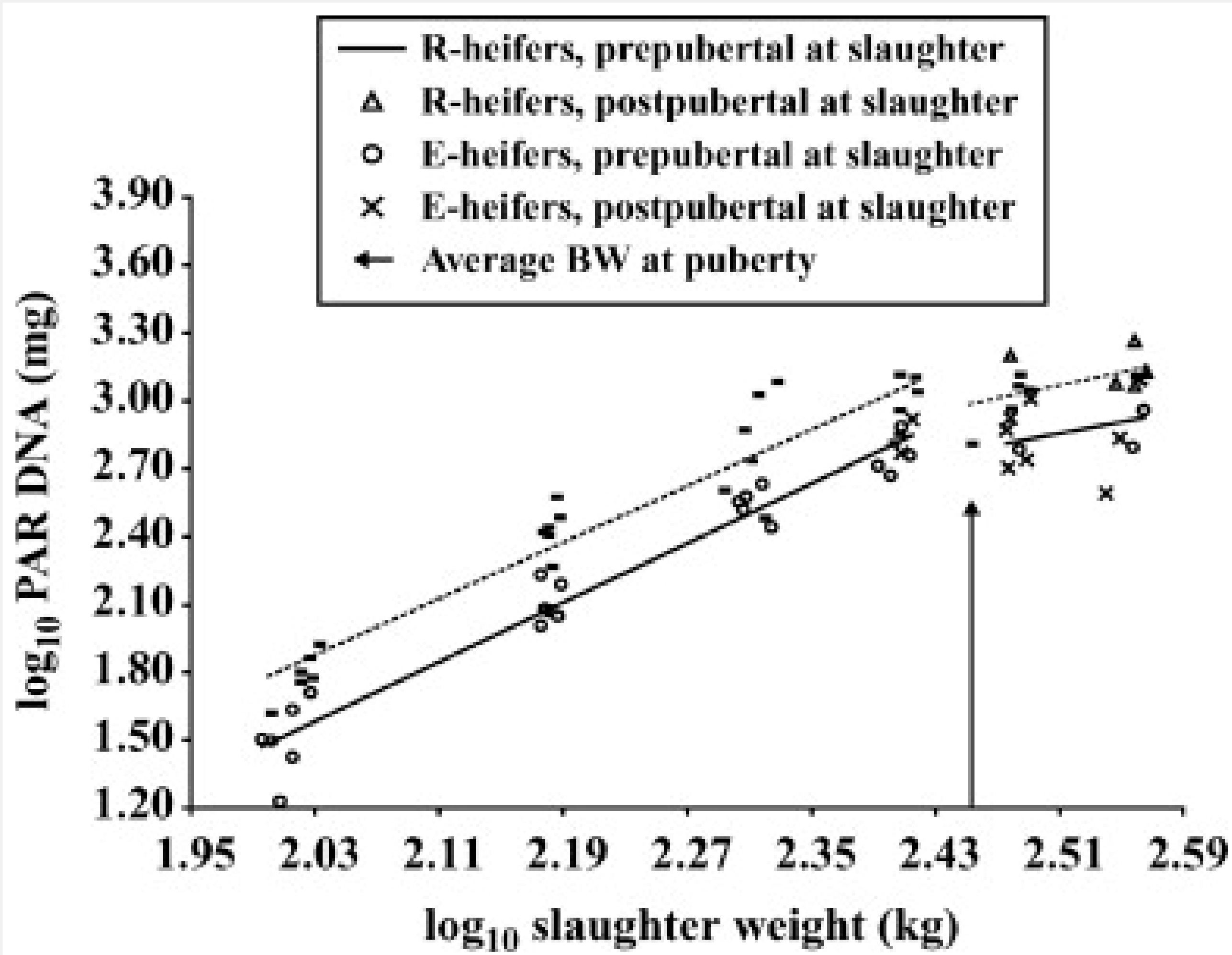

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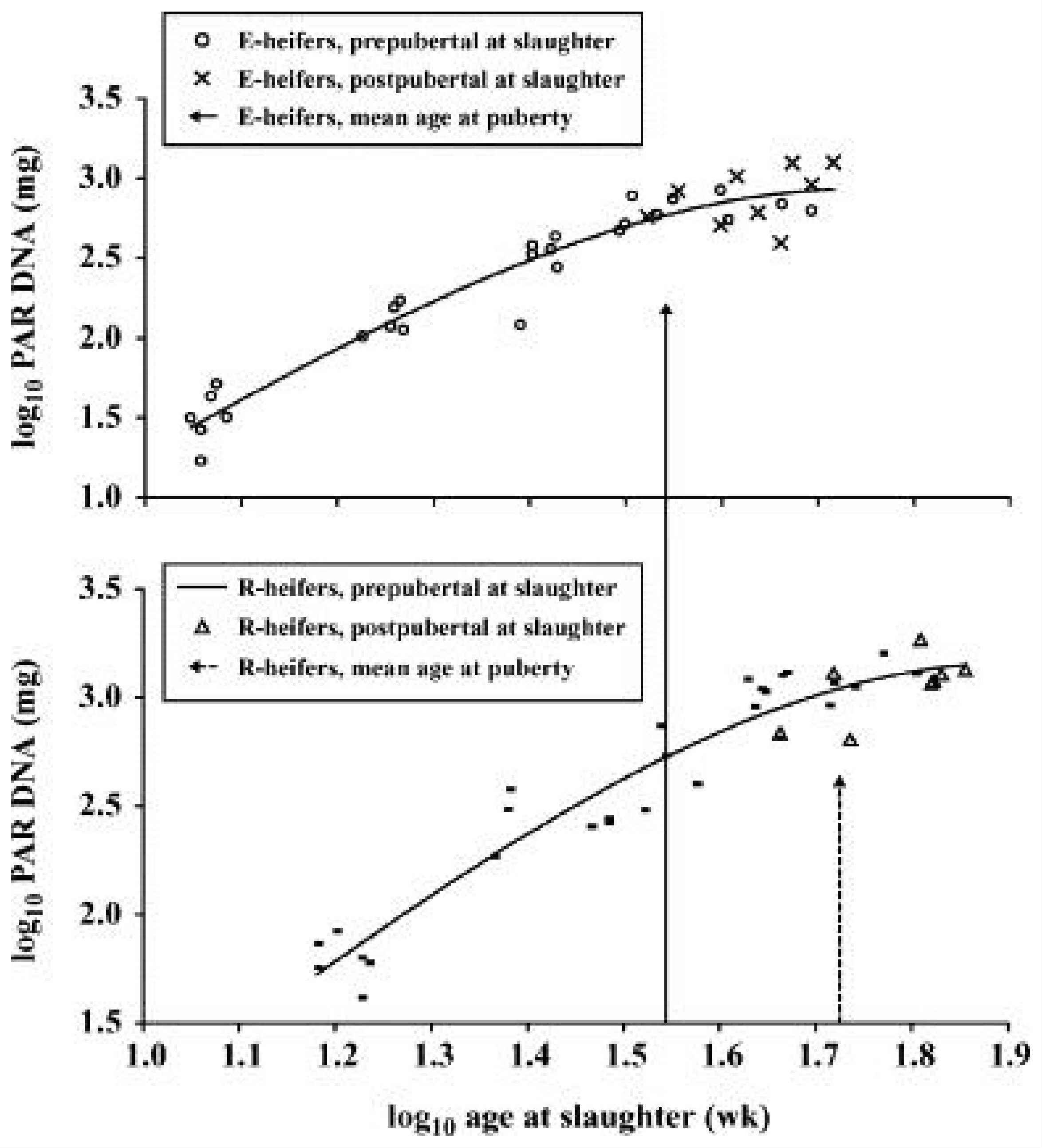
Source: Journal of Dairy Science 2006; 89:4298-4304 (DOI:10.3168/jds.S0022-0302(06)72476-6)

Figure 4



Source: Journal of Dairy Science 2006; 89:4298-4304 (DOI:10.3168/jds.S0022-0302(06)72476-6)

Mechanism behind uncoupling of the development of mammary parenchyma and reproductive organs

- Original hypotheis included GH
- Our data exclude direct effect of GH and circulating IGF-I
- Most convincing hypothesis
  - relate to effect of locally produced factors
    - IGFBP3 – Sejrsen et al., 2000
    - Leptin – indirect effect – Thorn et al.
    - TNF-alfa – conflicting data – Thorn et al
    - Other adipokines? i.e. adiponectin

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Mechanism behind uncoupling of the development of mammary parenchyma and reproductive organs

- Other interesting areas of research
  - **Stem cells** – Capuco et al. 2011, Animal
  - **Myoepithelial cells** – Ellis et al. 2012, J. Anim Sci.
  - **Adipose tissue/adipokines** – Hovey& Aimo 2010, J. Mam. Gl. Biol.Neopl.
  - **Gene expression** using laser dissection – Daniels et al. 2006, J Dairy Sci.

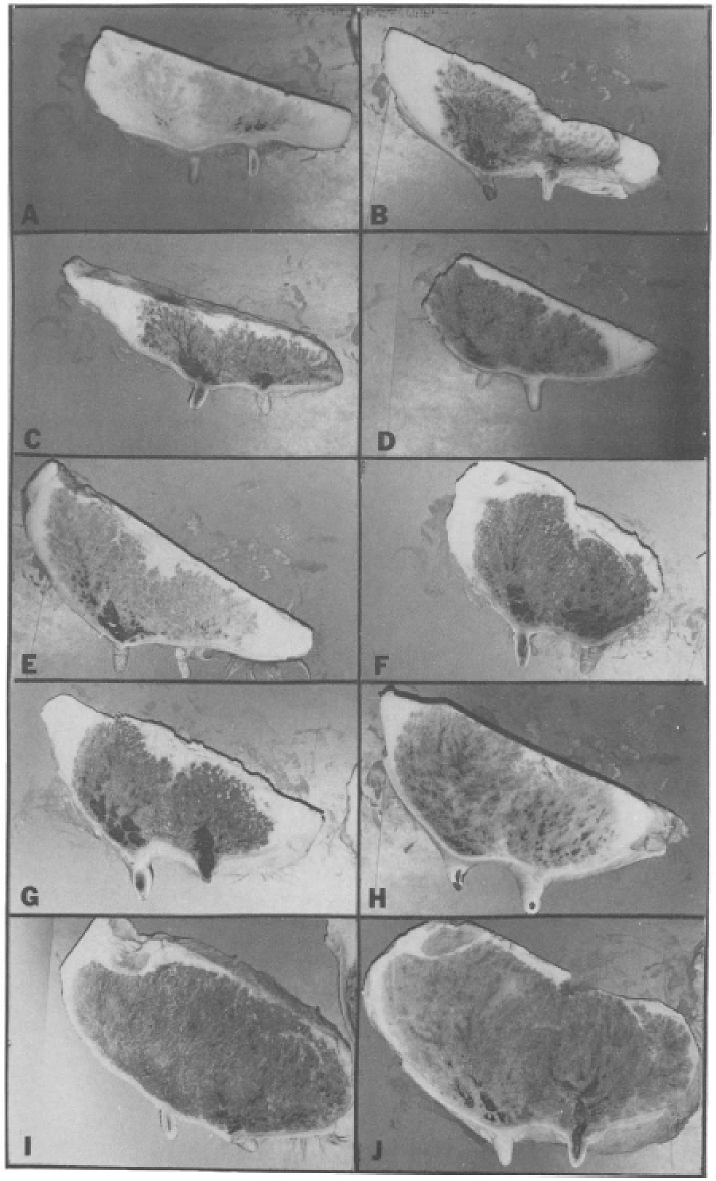
MAMMARY DEVELOPMENT AFTER PUBERTY

- No effect of nutrition after puberty
- Exponential growth during pregnancy

No uncoupling of development of mammary parenchyma and reproductive organ after puberty

Sejrsen et al. 1982 and Spicer et al 1984

	Postpubertal		
	M	H	Relative
Parenchyma, g	987	957	**97**
Uterus	202	205	**101**
Ovaries	16.6	18.7	**112**



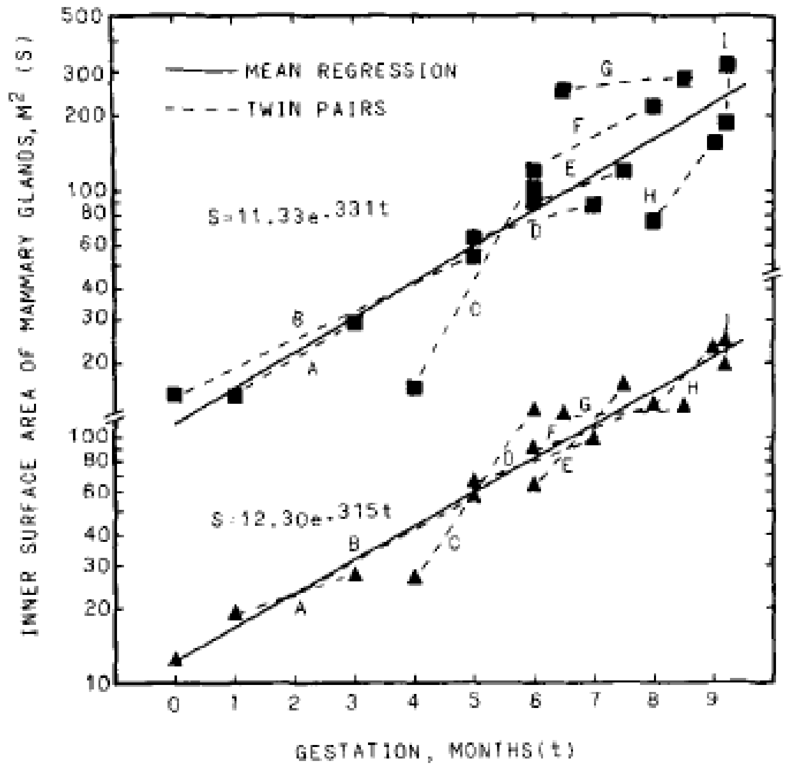


FIG. 3. The inner surface area (S) of alveoli and ducts of mammary glands from nine pairs of identical twin heifers according to stage of gestation. Upper data were adjusted only for differences in body weight. Lower data were corrected for both body weight and pair differences.

Swanson and Poffenbarger

MAMMARY DEVELOPMENT DURING LACTATION

- Cell proliferation
- Cell differentiation
- The shape of the lactation curve
- Effect of nutrition on cell turnover

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The influence of breed and parity on milk yield, and milk yield acceleration curves

J.V. Hansen [a,*], N.C. Friggens [b], S. Højsgaard [a]

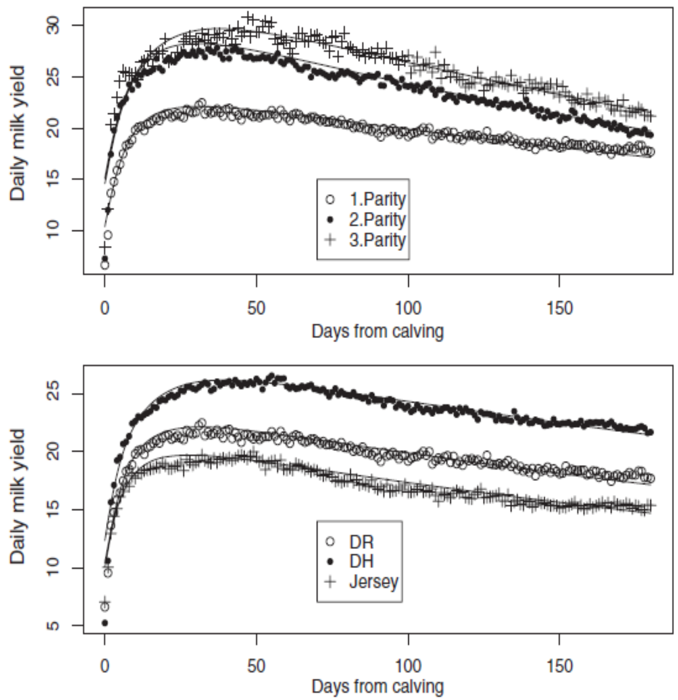


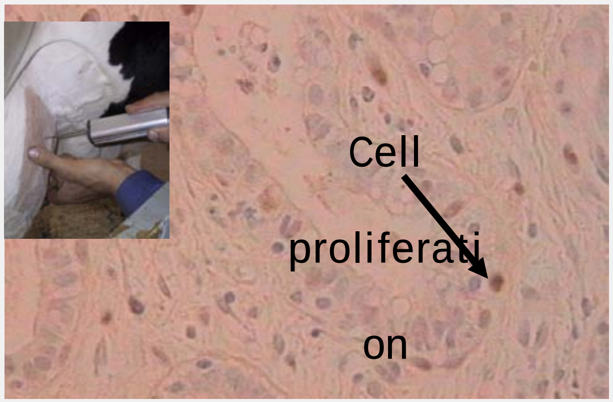
Fig. 2. Plots of mean daily milk yield. In the upper plot the mean daily milk yield is plotted for cows of breed Danish Red for each parity. In the lower plot the mean daily milk yield is plotted for cows of first parity for Danish Red, Danish Holstein and Jersey. Along each group of points the curve obtained by taking mean of the corresponding curve fits is drawn.

The shape of the lactation curve:

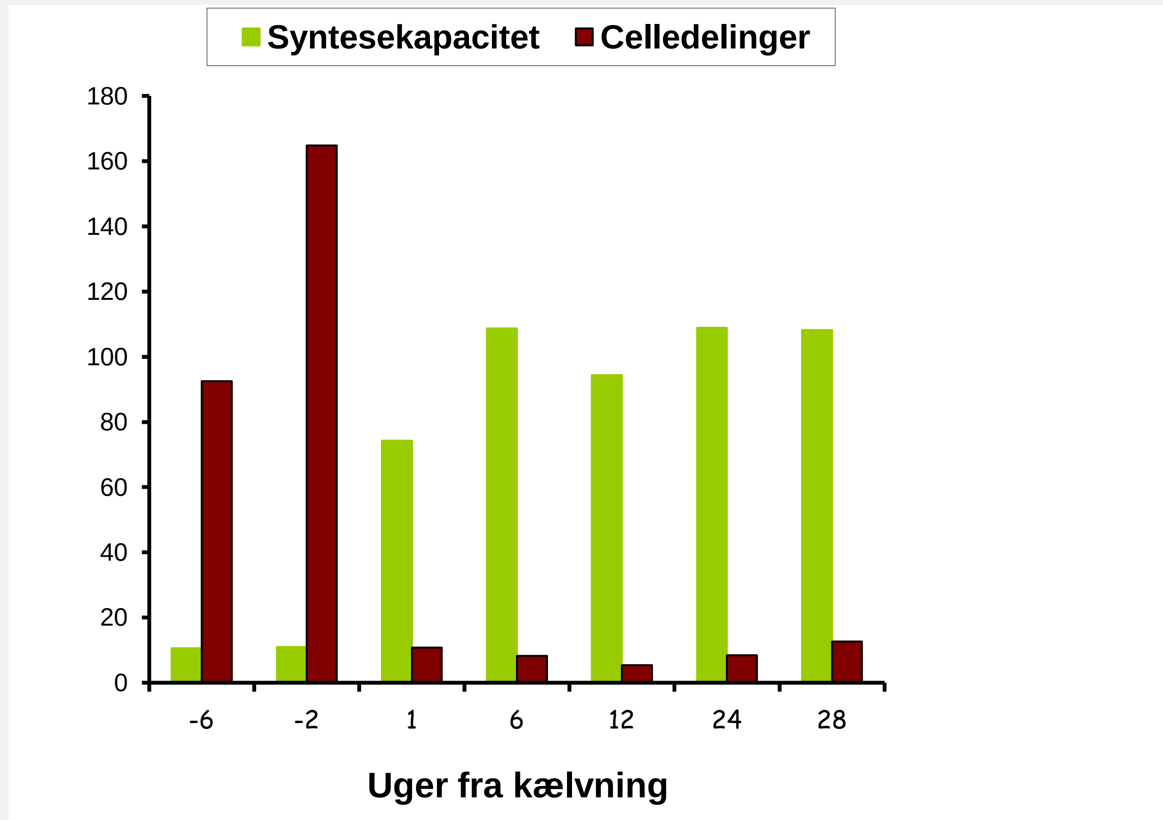
2 distinct phases:

- inccrease in yield w/o change in cell number!!
- after peak – mantinance of lactation

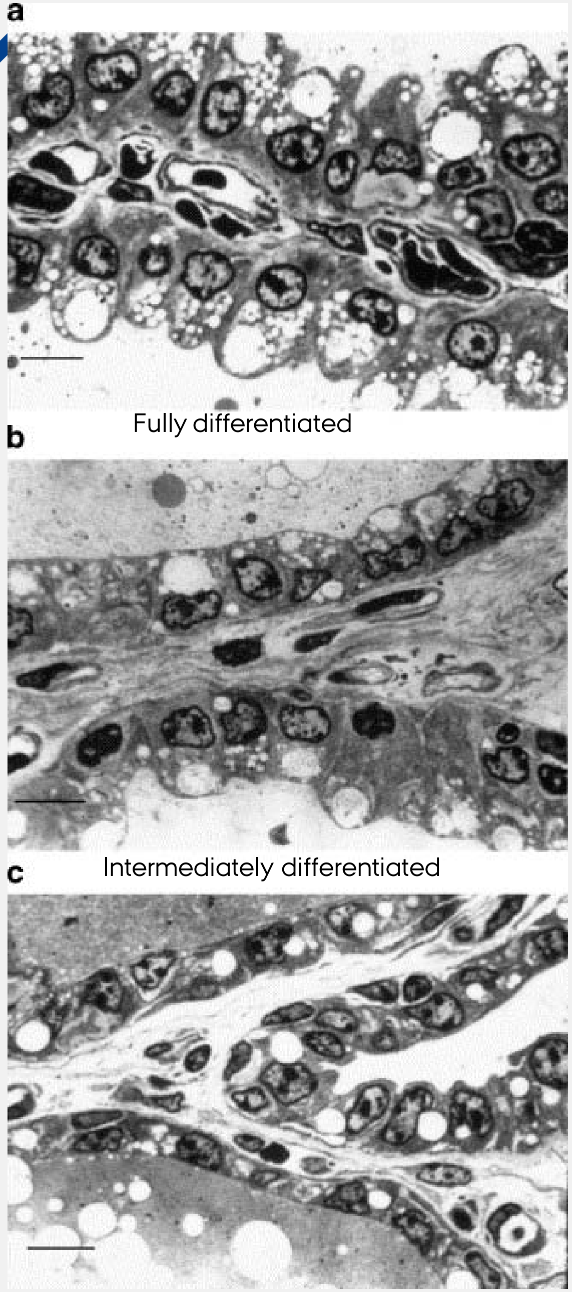
Cell proliferation declines sharply at calving in cows
Synthetic capacity increased gradually in early lactation



Confirms obervations in goats presented at the first BOLFA meeting by Chris Knight



Sørensen et al. 2006.



Fully differentiated

Intermediately differentiated

Poorly differentiated

Potential fates of mammary epithelai cells (MEC) after onset of lactation:

- Undifferentialted → full differentiation, / remain resting / undergo apoptosis

Questions?

- What determines the fate of the MEC?
- Can the fate of the MEC be changed?

Data on this by Annen et al.; Sina Safayi et al.

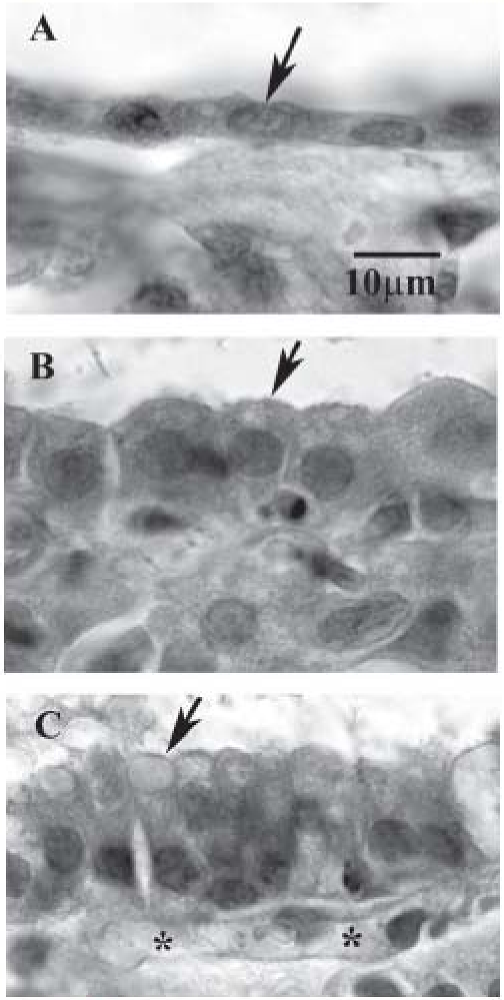


Figure 2. Light micrographs of mammary epithelial cells (MEC) at 3 developmental stages. A) poorly differentiated MEC; B) intermediately differentiated MEC; and C) highly differentiated MEC. The tissue sections are 4 to 5 μm thick and stained with periodic acid-Schiff stain. The location and the shape of the nuclei, the ratio of the cytoplasm and nuclei, and the abundance of secretory vesicles are different in the 3 developmental stages. A MEC is marked in each panel (arrow) and subepithelial capillary (*) including an erythrocyte is indicated in panel C. Magnification bar represents 10 μm.

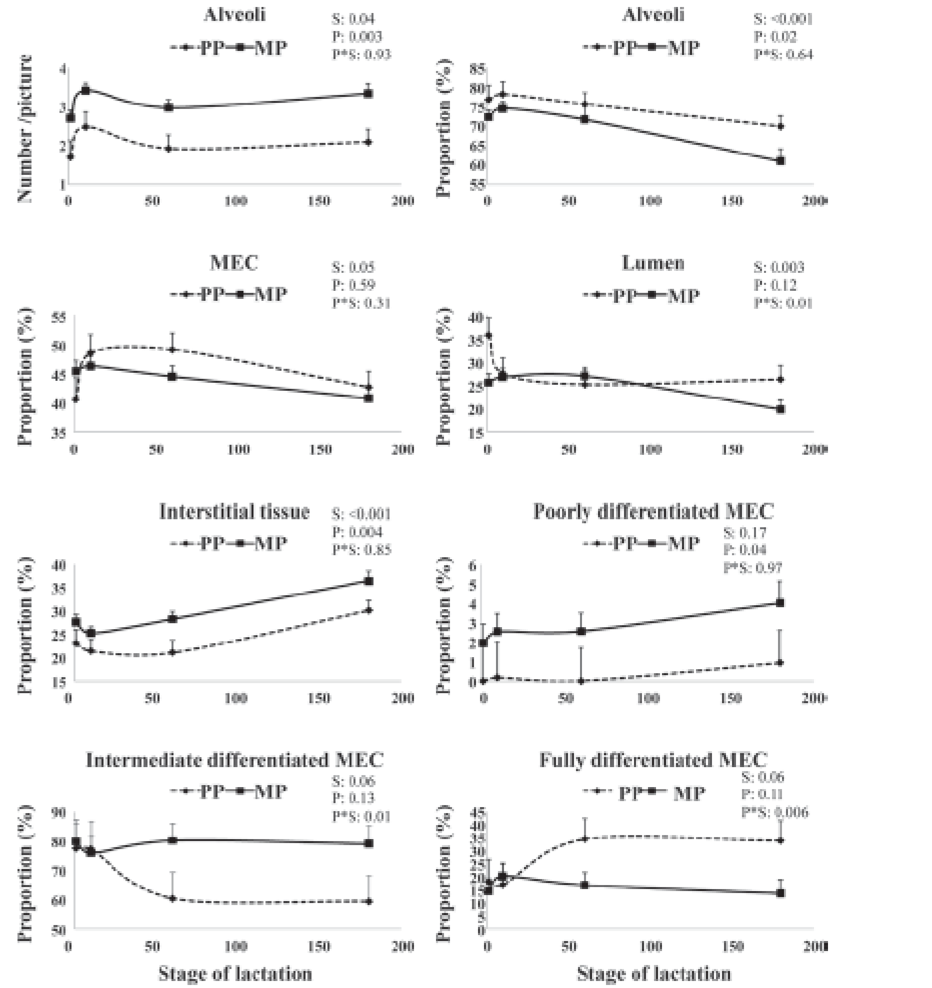


Figure 1. Number of alveoli per image, and the proportions (%) in mammary sections of alveoli, mammary epithelial cells (MEC), lumen, interstitial tissue, and poorly, intermediate, and fully differentiated MEC. PP and MP = primiparous (broken line) and multiparous (solid line) glands, respectively. *P*-values for stage of gestation-lactation (S), parity (P), and their interaction (P*S) are presented in each graph.

S. Safayi ,* P. K. Theil ,† L. Hou ,* M. Engbæk ,* J. V. Nørgaard ,† K. Sejrsen ,† and M. O. Nielsen *, 2011. J. Dairy Sci. 93 :203–217

S. Safayi ,* P. K. Theil ,† V. S. Elbrønd ,* L. Hou ,* M. Engbæk ,* J. V. Nørgaard ,† K. Sejrsen ,† and M. O. Nielsen 2010. J. Dairy Sci. 93 :1478–1490

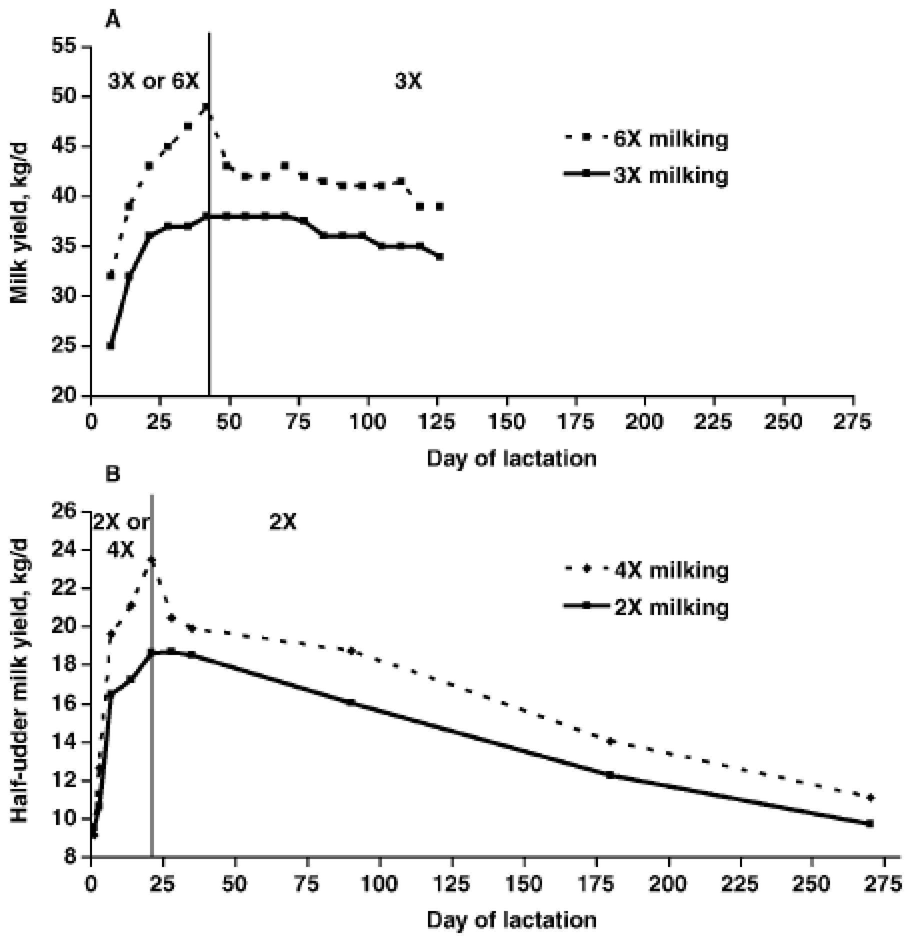


Figure 2. Panel A. Lactation curves of multiparous cows milked six times daily (6X) for d 1 to 42 of lactation or thrice daily (3X) for the entire lactation (redrawn from Bar-Peled et al., 1995; used by permission from the Journal of Dairy Science; 78:2726–2736). Panel B. Lactation curves of multiparous cows milked 4 times daily (4X) unilaterally for d 1 to 21 or twice daily for the entire lactation (2X; from E. H. Wall and T. B. McFadden, unpublished results). Vertical lines indicate cessation of frequent milking.

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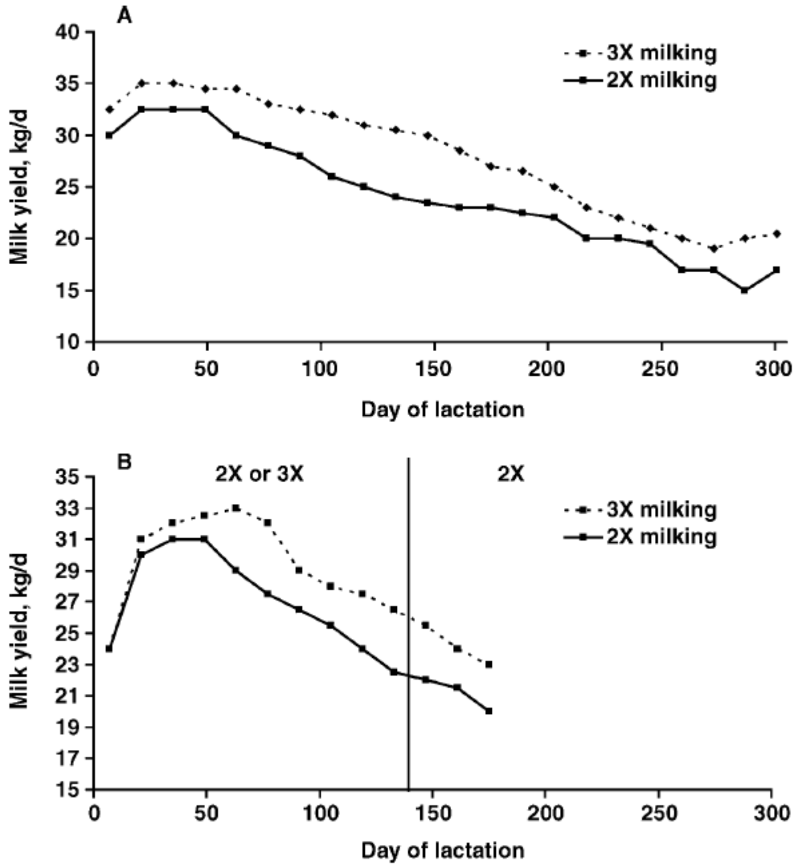

Figure 1. Panel A. Lactation curves of multiparous cows milked twice (2X) or thrice (3X) daily for the entire lactation (redrawn from Amos et al., 1985; used by permission from the Journal of Dairy Science; 68:732–739). Panel B. Lactation curves of multiparous cows milked 2X or 3X for the first 143 d of lactation followed by 2X for the remainder of lactation; the vertical line indicates the cessation of frequent milking (redrawn from Pearson et al., 1979; used by permission from the Journal of Dairy Science; 62:1941–1950).

Effect of bovine somatotropin (bST) on milk yield and voluntary intake
(after Bauman et al, 1985).

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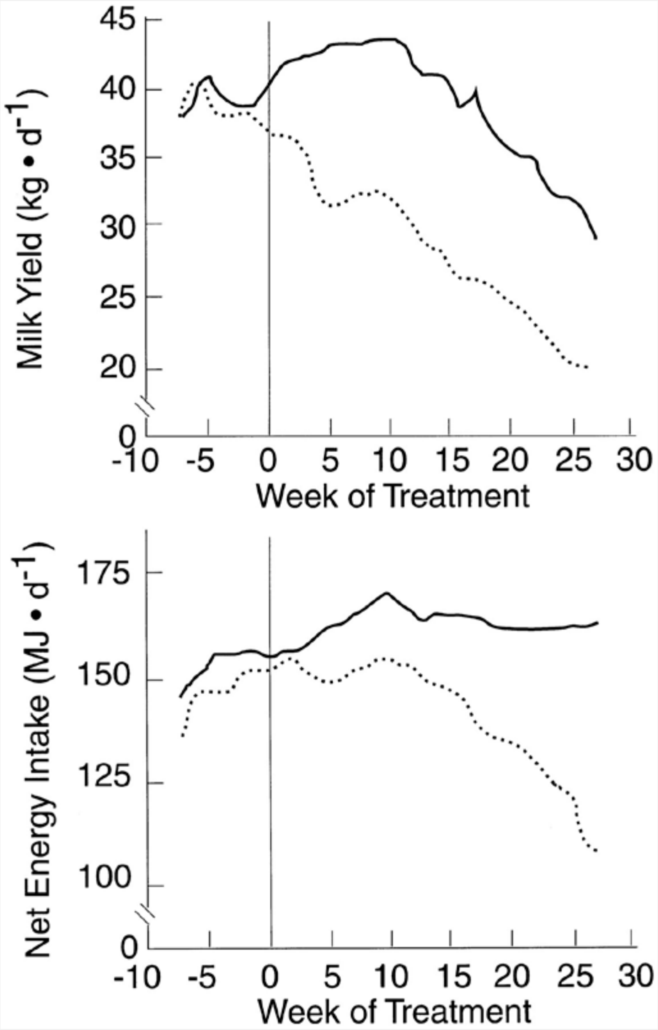

ETHERTON T D , BAUMAN D E Physiol Rev 1998;78:745-761

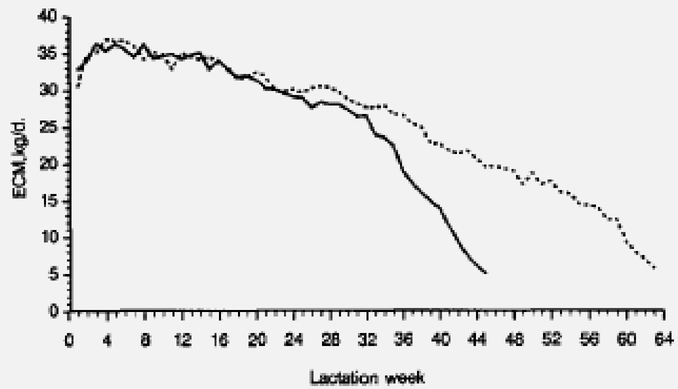

Fig. 1. Average daily yield of energy corrected milk (ECM) during the first experimental lactation in herd 2 (40% primiparous, 60% multiparous) for cows managed for 12- (————) or 18- (- - -) month calving intervals.

Bertilsson et al., 1997

Mammary cell turnover and pregnancy

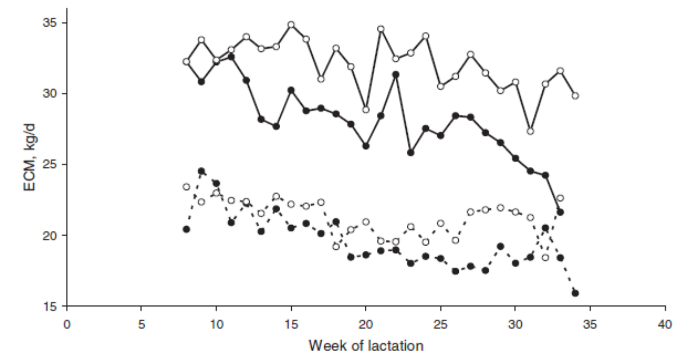

Figure 1 Average daily energy-corrected milk yield (ECM) of non-pregnant cows (open circle), and pregnant cows (closed circle) inseminated week 12 (day 88 ± 9 (mean ± SEM)) after parturition on either normal (full line) or low (dotted line) feeding level. Biopsies were taken at week 27 (day 189 ± 3) of pregnancy, i.e. week 39 (day 276 ± 10) of lactation for pregnant cows and week 32 (day 224 ± 3) of lactation for non-pregnant cows. $N = 16$. Values are least squares means.

J. V. Nørgaard, M. T. Sørensen-, P. K. Theil, J. Sehested and K. Sejrsen. Animal (2008), 2, 588–594

Models for cell turnover during lactation

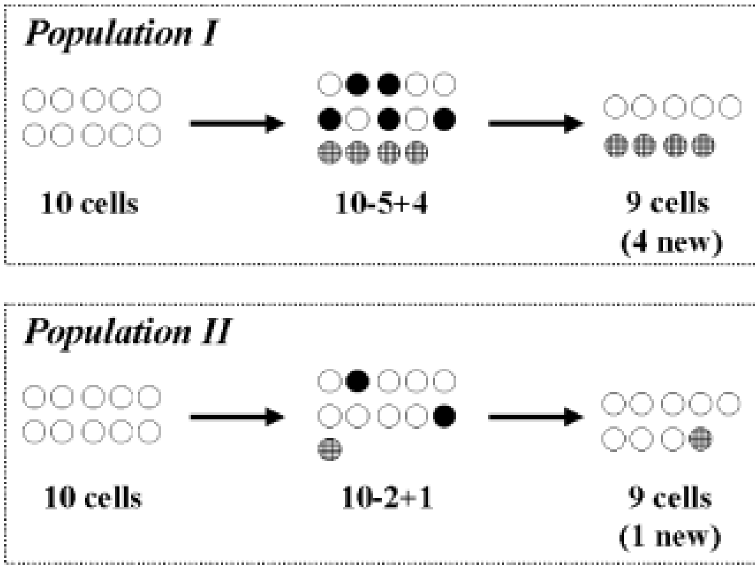

Figure 1. Rates of cell proliferation and cell death determine the net gain or loss of cells and extent of cell renewal within the population. Depicted is a population of cells undergoing net regression. Open circles represent the initial populations of cells; cross-hatched circles depict new cells formed by cell proliferation; and cells that die during this period are depicted by black circles. In both panels, the net loss is identical because the difference between rates of cell death and proliferation are one cell. However, cell renewal differs markedly between panels. In the upper panel, the population of nine cells contains four new cells. In the lower panel, the population of nine cells contains one new cell. (Capuco et al., 2001a)

A. V. Capuco, D. L. Wood, R. Baldwin,
K. Mcleod, and M. J. Pape. 2001. J. Dairy Sci. 84:2177–2187

- ● Proliferation cells
- ○ Apoptotic cells

Net gain/ loss of cells:

determined by the balance between rates of cell proliferation and rates of apoptosis

Cell renewal:

determined by the relative amount of cells proliferating and undergoing apoptosis.

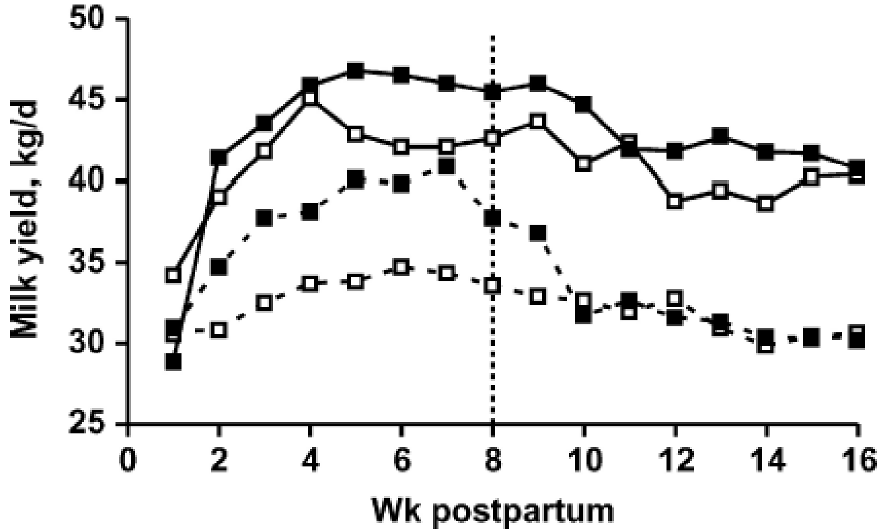

Figure 1. Milk yield during the experimental period, where cows were milked either 2 times daily (2×, open squares) or 3 times daily (3×, filled squares) and fed either low- (dotted lines) or high-energy density diets (solid lines). The vertical line represents the time after which all cows were milked 2 times daily.

Mammary epithelial cells undergoing apoptosis or proliferation in biopsies taken from cows milked either 2 times daily (2×) or 3 times daily (3×) and fed either low or high energy density diet

	Experimental group				*P* value		
	2× low	3× low	2× high	3× high	Milking frequency	Energy density	Frequency × Energy
Apoptosis. %	0.20	0.05	0.24	0.16	0.32	0.55	0.70
Proliferation, %	0 …	0.04	0.16	0.30	0.20	0.001	0.32

Nørgaard JV, Sørensen A, Sørensen MT, Andersen JB and Sejrsen K, 2005. Journal of Dairy Science, 88, 975-982.

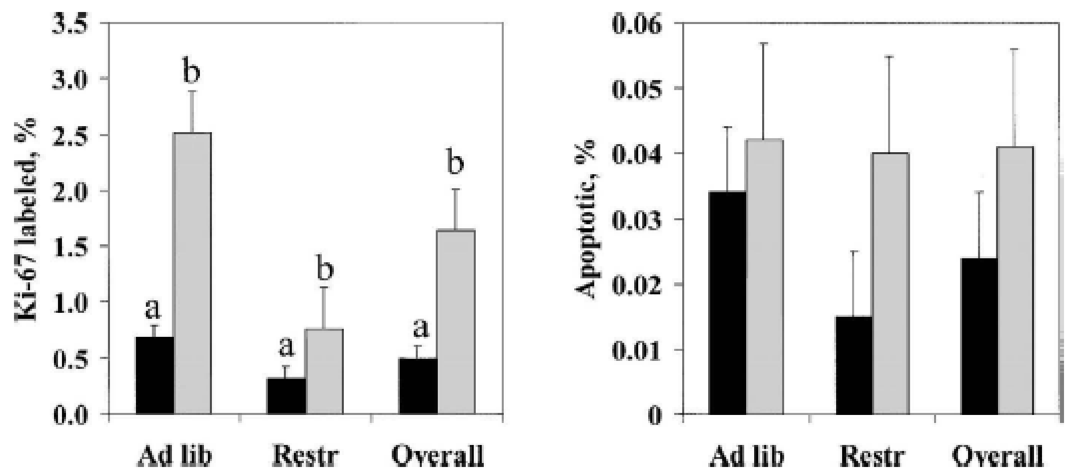

Figure 5. Effect of bST on mammary cell proliferation and apoptosis. Six cows were fed ad libitum (Ad lib) and six cows were restricted to 80% of their ad libitum intake (Restr). Values shown are for the control period (black bar) and bST treatment period (gray bar) for each dietary group and the overall effect (12 cows). Left panel Ki-67 labeling index, expressed as a percentage of total cells. Right panel: apoptotic index, expressed as a percentage of total cells. Each bar represents the mean ± SE for six cows per dietary group and 12 cows for overall effect. Within a dietary category, means without a common superscript differ ($P < 0.05$). Effect of diet on Ki-67 expression was significant ($P < 0.05$). Neither diet nor bST altered the apoptotic index ($P > 0.05$).

A. V. Capuco, D. L. Wood, R. Baldwin,

K. McLeod, and M. J. Paape. 2001. J. Dairy Sci. 84:2177-2187

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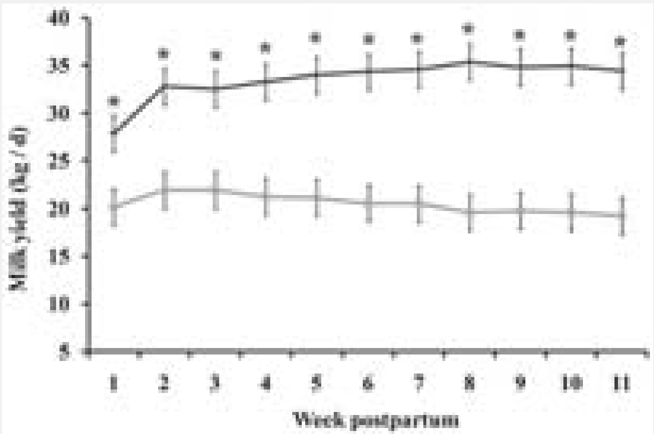

Figure 1. Effects of feeding level on milk yield during the first 11 wk of lactation in restrictively or normally fed dairy cows (n = 8 in each group). The basal-diet group is in dark gray and the restricted-diet group is in light gray (* indicates significant difference at $P < 0.001$).

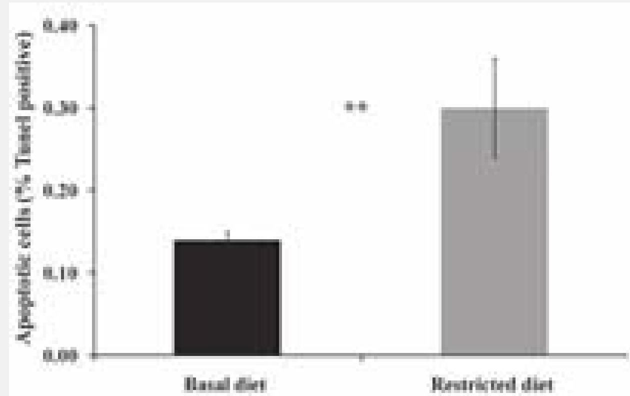

Figure 4. Effects of feeding level on apoptosis in mammary extracts after 11 wk postpartum in restrictively or normally fed dairy cows (n = 8 in each group). (A) 6-Diamidino-2-phenylindole (DAPI) staining (1 and 2) and terminal deoxynucleotidyl transferase 2'-deoxyuridine, 5'-triphosphate (dUTP) nick end labeling (TUNEL) staining (3 and 4) in mammary gland tissue sections from cows fed with a basal diet (left panels) or a restricted diet (right panels). Arrowheads indicate TUNEL-positive nuclei. (B) Apoptosis rates were assessed by quantification of TUNEL nuclear staining in tissue sections (**$P < 0.01$). Color version available in the online PDF.

Dessauge et al. 2011. Journal of Dairy Science , 94,

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CONCLUDING REMARKS

- Research in mammary offers:
  - A barnyard of opportunities

- Recognize the collaboration with many collegues

- A special recognition of the contribution of Tony Capuco