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Gonadotrophin secretion in the GnRH challenged prepuberal beef heifer and its relationship to the follicular numbers and body weight

Michael S. Rowland

To the Graduate Council:

I am submitting herewith a thesis written by Michael S. Rowland entitled "Gonadotrophin secretion in the GnRH challenged prepuberal beef heifer and its relationship to the follicular numbers and body weight." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Bert H. Erickson, Major Professor

We have read this thesis and recommend its acceptance:

J.D. Smalling, W.R. Backus, J.C. Waller

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Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

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B H Erickson

Bert H. Erickson, Major Professor

We have read this thesis
and recommend its acceptance:

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Date May 26, 1994

GONADOTROPHIN SECRETION IN THE GnRH CHALLENGED PREPUBERAL BEEF HEIFER AND ITS RELATIONSHIP TO FOLLICULAR NUMBERS AND BODY WEIGHT

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Michael S. Rowland

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Study I

Correlations of Gonadotrophin Concentrations in peripheral blood with Follicular Number and Body Weight in the Beef Heifer

ABSTRACT

To examine the extent to which gonadotrophin concentration in peripheral blood is correlated with follicular numbers and body weight [birth, weaning (ADJ205) and yearling], prepuberal Angus (n=30) and Crossbreds (n=22) heifers were evaluated at 5, 7, 9, 11 and 14 mo of age. Basal and GnRH-stimulated gonadotrophin secretion was determined from the average of two bleedings effected at 1.5 hour intervals. Gonadotrophins (LH & FSH) were quantified by RIA. All heifers were synchronized and in luteal phase when ovariectomized at 16 mo of age. Ovaries were sectioned and stained for light microscopy.

GnRH-stimulated gonadotrophin secretion changed with age. The FSH response increased significantly between 5 and 9 mo ($p < 0.01$) and then decreased at 14 mo ($p < 0.01$). LH release increased with age and concentrations were lower at 5, 7 and 9 mo than at 11 and 14 mo ($p < 0.01$). Basal concentrations of FSH and LH did not change between 5 and 14 mo ($p > 0.10$). However, FSH did show a downward trend as age increased.

Basal and-GnRH stimulated concentrations of FSH were not associated with follicular numbers ($p > 0.10$). However, when heifers were partitioned according to their gonadotrophin concentrations (low, medium or high), associations between normal vesicular, atretic vesicular and total vesicular follicles and concentration of FSH were significant at 14 mo

for basal and 7 mo for GnRH-stimulated secretion. Groups varying in FSH concentration were not different in follicular numbers at any age ($p > 0.10$). In contrast, basal and GnRH-stimulated LH groups were significantly different in number of primary follicles at 14 mo ($p < 0.06$). Additionally, basal LH was positively related to number of primary and secondary in the low ($r= .68$, $p < 0.03$) and high groups ($r= .56$, $p = 0.08$), and number of secondary follicles was negatively correlated with GnRH stimulated LH secretion in the high group ($r= -.64$, $p < 0.05$).

Body weight was inversely related to basal FSH concentration at 9, 11 and 14 mo and GnRH-stimulated secretion at 5, 7 and 9 mo. In contrast, GnRH-stimulated LH was positively associated with weaning and yearling weight at all ages except 9 and 14 mo, but basal LH was not associated with body weight ($p > 0.10$). Basal and GnRH-stimulated FSH groups were not different in body weight at any age ($p > 0.10$). With LH, however, the high responders to GnRH at 5, 11 and 14 mo were heavier as yearlings ($p < 0.10$).

The results suggest that the combination of yearling weight and basal and GnRH-stimulated concentrations of gonadotrophins in peripheral blood could be useful indicators of number of follicles and this combination might be useful in identifying heifers of superior reproductive potential.

INTRODUCTION

Function and Control of Gonadotrophins in the Female Bovine

The master gland of the endocrine system is the hypothalamus. The hypothalamus is formed from part of the wall of the third ventricle of the brain and is connected with the pituitary by means of the median eminence and infundibulum (Norman and Litwack, 1987). Among other hormones gonadotrophin releasing hormone (GnRH) is synthesized and secreted by the hypothalamus. GnRH is a decapeptide (McCann and Ojeda, 1992) which directly acts upon the gonadotrophs of the anterior pituitary to stimulate the release of two 30,000 molecular weight (McCann and Ojeda, 1992) protein hormones: Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). The synthesis and release of GnRH is modified by neurotransmitters (Opioids:Leshin et al.,1991, Malven et al., 1986; Catecholamines: Chappel, S.C., 1985, Hardin & Randel, 1983; Indoleamines: Dailey et al., 1987, Vitale et al., 1986; amino acids: Estienne et al., 1990) secreted by neurons in the forebrain, midbrain and hindbrain and by steroids produced by the gonads (Fink, 1988), adrenals, brain and certain peripheral tissues. Estrogens, progestins and androgens produced by the theca interna, granulosa and corpus luteum of the ovary influences GnRH secretion. During the follicular and luteal phases of the estrous cycle, 17β-estradiol (E_2) and

progesterone (P_4), respectively inhibit the release of GnRH and consequently the release of FSH and LH. This is known as negative feedback control. However, at the end of follicular phase (d 20-21 of the estrous cycle) when a dominant follicle is producing high levels of E_2, the E_2 has a stimulatory effect on the release of GnRH and this GnRH plus increased sensitivity of the pituitary to GnRH subsequently causes the preovulatory surge of gonadotrophins (Kesner et al., 1981). This is known as positive feedback control.

The primary functions of FSH are the initiation and support of follicular growth, induction of the theca interna and stimulation of the production of E_2 and inhibin (Dahl and Hseuh, 1988). Additionally, FSH has the ability to upregulate its own receptors, and synergizes with E_2 to induce LH receptors in thecal cells. Inhibin is a protein produced by the granulosa cells of advanced follicles which selectively inhibits the release of FSH (Dahl and Hsueh, 1988). Inhibin is thought to be involved in the selection of the dominant follicle which occurs between days 7 and 11 (Knopf et al. 1989) and days 16 and 17 (Pierson and Ginther, 1988) in a two follicular wave estrous cycle. It is believed that of the cohort of follicles recruited for ovulation the larger, more mature follicle will begin to produce inhibin and E_2, thus, causing the reduction in FSH that leads to the atresia of large subordinate vesicular follicles (Spicer et al., 1986; Ireland, 1983). Important functions of LH are ovulation,

stimulation of P_4 synthesis and secretion, androgen production by the thecal cells and differentiation of granulosa cells prior to ovulation and formation of the corpus luteum (CL). Structurally, both FSH and LH are comprised of α and β-subunits. They both contain the same α-subunit, but have a different β-subunit. Thus, the specificity of the hormone is denoted by the β-subunit (Dahl and Hsueh, 1988).

Prepuberal Gonadotrophin Concentration in the Heifer

An early study by Desjardins and Hafs (1968) characterized the pituitary content of FSH and LH in the Holstein heifer from birth to 12 months of age. Pituitary content of FSH was highest at 1 mo (2.7 μg) then decreased at 2 mo (1.1 μg) and remained relatively unchanged through 12 mo of age. Pituitary concentrations of LH increased from birth and was highest at 7 mo (10.19 μg) and then declined to 6.79 μg at 12 mo of age. Scham et al. (1981) reported that plasma concentrations of LH increased from early calfhood (4 mo) until puberty (11 mo). Dodson et al (1988) also found that mean plasma concentrations of LH increased to 9 months of age. This increase is a probable result of a decreased inhibition of 17β-estradiol on LH secretion (Day et al., 1987; Schillo et al., 1982). In contrast, McLeod et al. (1984) and Kiser et al. (1981) found that basal LH concentration was similar in heifers of 4 and 10 months of age. Concentrations

of FSH tended to rise and fall in parallel with LH during the prepuberal period (Scham et al., 1981). However, unlike LH, prepuberal concentrations of plasma FSH have been found to be similar to that of the cycling cow (McLeod et al., 1984; Akbar et al., 1974).

The ability of heifers to respond to exogenous GnRH which stimulates secretion of gonadotrophins occurs at an early age. Barnes et al. (1980) reported that the pituitary response to GnRH (200 μg) at 3 months of age is no different than that at either 6 or 9 months of age. Furthermore, McLeod et al. (1984) also reported that the LH response to 5 μg of GnRH at 4 and 10 mo of age was not significantly different. In contrast, GnRH stimulated plasma concentrations of FSH were only evident at 4 mo and not at 10 mo with 5 μg injection of GnRH. Data cited by McLeod et al. (1984) suggested that the sensitivity of the pituitary to low doses of GnRH in the prepubertal animal increases with increasing age. This is a probable result of the hypothalamic-pituitary complex becoming less sensitive to 17β-estradiol negative feed back as the heifer ages (Schillo et al., 1982). Finally, McLeod et al. (1984) also showed that the threshold dose of GnRH for FSH is much higher than that necessary to evoke a LH response, suggesting that FSH, to a lesser degree, is not as dependent upon GnRH as is LH.

Follicle Populations in the Bovine Ovary

Follicles exist in varying states of development in the ovary and these are designated, respectively as primary, growing and vesicular. The primary follicle, composed of a oocyte and one layer of granulosa cells, is the most immature state and exists in the largest number. The number of primary follicles for the beef heifer is around 133,000 and this number remains relatively constant until around four years of age. Thereafter numbers diminish rapidly and approach zero in cows of 15 to 20 years of age (Erickson,1967). The growing follicle, composed of a oocyte and two or more layers of granulosa cells, is derived from the primary follicle. Mean number of growing follicles reaches a high point at 4 months and then gradually decreases as the population of primordial follicles is depleted (Erickson, 1967). The vesicular follicle arises from the growing follicle. Number of vesicular follicles increase as the number of growing follicles increases, but remains high until the cow reaches 10- to 14-years of age (Erickson, 1967).

Justification and Objectives

At present, no research has been done relating number and class of ovarian follicles and body weight to concentrations of follicle stimulating hormone (FSH) and luteinizing hormone

(LH) in the peripheral blood of the bovine. Additionally, changes in basal and GnRH stimulated concentrations of FSH and LH have not been clearly defined. Therefore, the objectives of this study were (1) determine the changes in basal and GnRH stimulated secretion of FSH and LH that occur with increase in age, (2) determine if number and class of ovarian follicles is related to basal and GnRH stimulated secretion of the gonadotrophins at various ages, and (3) determine if body weight is related to basal and GnRH stimulated secretion of FSH and LH at various ages.

MATERIALS AND METHODS

Experimental Design

A three year study was conducted using fifty-two beef heifers (30 Angus and 22 Crossbreds). Heifers were evaluated at ages ranging from 5 months to 14 months.

The response of the pituitary to GnRH was tested by means of the analog des-Gly^{10}, [$_{D}$Ala^{6}] ethylamide. The potency of the analog is approximately 50 times that of the native GnRH. Five μg were injected intramuscularly in the 5 to 7 month heifer and 10 μg were injected at older ages. Gonadotrophin concentrations were quantified via blood samples collected at 0, 1.5 and 3 hours after the injection of GnRH. The zero plus additional bleedings were used to establish basal concentrations. GnRH stimulated gonadotrophin secretion was estimated from the total of 1.5 and 3 hours bleedings.

All blood samples were collected via serum separation tubes (Vacutainer) from the non-catheterized jugular vein. They were immediately placed on ice and centrifuged at 3,000 rpm for 20 minutes 3 hours later. Serum was then poured into storage tubes and stored at -20°C until the gonadotrophins could be measured by Radioimmunoassay (RIA). All heifers were weighed at birth, weaning (ADJ 205) and approximately 1 year of age. Individual body weights can be found in Appendix C.

Histological Technique

All 52 beef heifers (20 from year 1, 18 from year 2 and 14 from year 3) were synchronized and in luteal phase when bilaterally ovariectomized via an incision in the paralumber fossa of the left side under local and spinal anesthesia at 16 months of age. Ovaries were measured (cm), sliced into quarters, fixed in Bouin's fluid, sectioned at 10 μm and stained with Hematoxylin and Eosin (H&E). Number of primary, growing and normal vesicular and atretic vesicular follicles were determined by light microscopy in two sections from each ovarian quarter. Sections were separated by 100 μm.

The primary follicle is characterized by the presence of an oocyte and a single layer of granulosa cells, and was counted only when the nucleus of the oocyte could be seen. The growing follicle is distinguished from the primary follicle by the layers of granulosa cells surrounding the oocyte. A follicle was defined as growing when the oocyte was encompassed by two or more layers of granulosa cells. Vesicular follicles were characterized by a clearly defined cavity and were classified as either normal or atretic. Normal vesicular follicles had healthy granulosa cells, while atretic vesicular follicles have either necrotic or no granulosa cells. A granulosa cell was considered necrotic when it was detached from the remaining layers or had a nucleus which was pycnotic.

Radioiodination Procedures

The Chloramine-T method described by Brown et al. (1983) was used for radioiodination of luteinizing hormone (LH). LH for standards and iodination (USDA bLHI-1) was provided by Dr. L.H. Reichart, Albany Medical School, New York.

The Iodogen technique was used to radioiodinate follicle stimulating hormone (FSH) (Bolt and Rollins, 1983). FSH for standards and iodination (USDA bFSH I-2) was provided by Dr. D.J. Bolt, USDA Beltville, Maryland.

Iodination procedures were previously described by Hillyer (1992).

Radioimmunoassay Technique

Follicle stimulating hormone was quantified using a modified version of Brown et al. (1983). A standard reference curve (0.015 to 4.0 ng/200μl) was constructed using triplicate samples of the standard reference protein (USDA bFSH I-2). Antiserum (USDA S0122 bFSH beta) was provided by Dr. D.J. Bolt, USDA Beltsville Maryland. Inter-assay variation was determined by means of the results of 29 assays of a common pooled sample. Intra-assay variation was <5% and inter-assay variation was 10%. Details for FSH procedure can be found in Appendix B.

Luteinizing hormone concentrations were quantified using

the method by Brown et al. (1983). A standard reference curve (.03 to 4.0 ng/200μl) was constructed using triplicate samples of the reference LH USDA bLH I-1. Antiserum (USDA-309-684$_p$) was provided by Dr. D.J. Bolt. Interassay variation was determined from 35 assays as mentioned above for FSH. Intra-assay variation was <5% and inter-assay variation <7%. Details for LH procedure can be found in Appendix B.

Statistical Analysis

Least square means, standard errors of means, standard deviations and means were done by General Linear Model (GLM). Correlations between gonadotrophins and ovarian follicles and body weight were computed in GLM by SAS.

RESULTS

Follicle Stimulating Hormone (Age Effects)

Basal Secretion

No significant difference between ages in basal secretion was found. However, there was a downward trend as age increased (Fig 1).

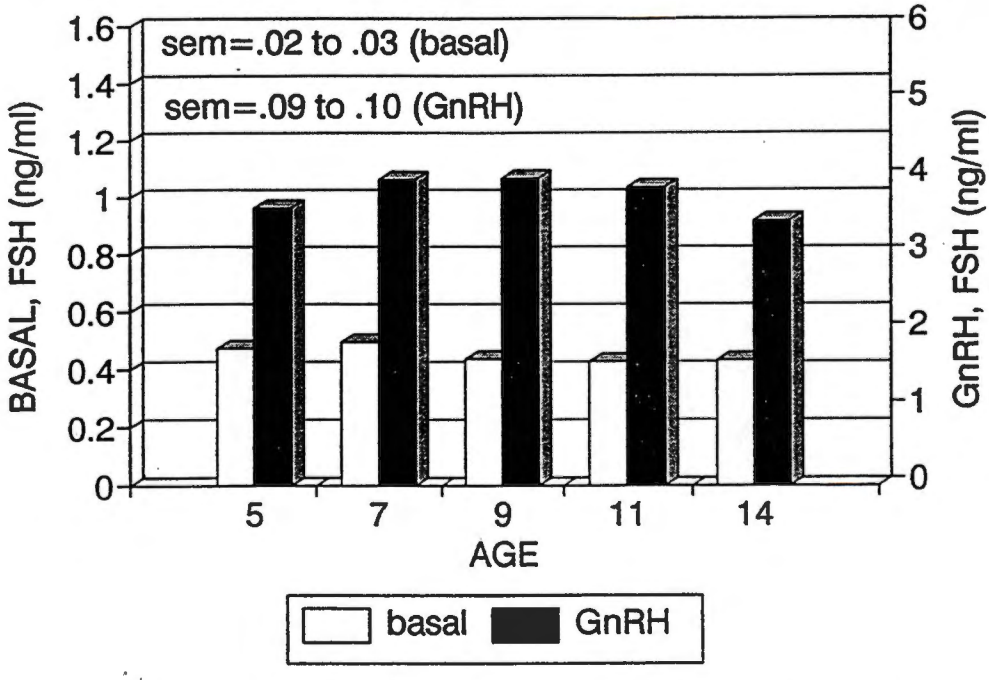

Fig 1 Age-related basal and GnRH-stimulated FSH concentration in the beef heifer (5-14 mo).

GnRH Response

GnRH-stimulated secretion increased from 5 mo and was highest at 9 mo ($p < 0.01$; Fig 1). Thereafter, GnRH stimulated secretion diminished and was significantly lower than the 9-month value at 14 mo ($p < 0.01$). Additionally, GnRH stimulated secretion at 14 mo was not different from that at 5 mo ($p > 0.10$).

Luteinizing Hormone (Age Effect)

Basal Secretion

No significant age effect was found in basal secretion ($p > 0.10$; Fig 2).

GnRH Response

Pituitary responsiveness to GnRH increased with age and the difference between 5, 7 and 9 months and 11 and 14 months was significant ($p < 0.01$; Fig 2). Differences between 5, 7 and 9 mo were not significantly different but the response at these ages was significantly lower than that of 11 and 14 mo ($p < 0.01$).

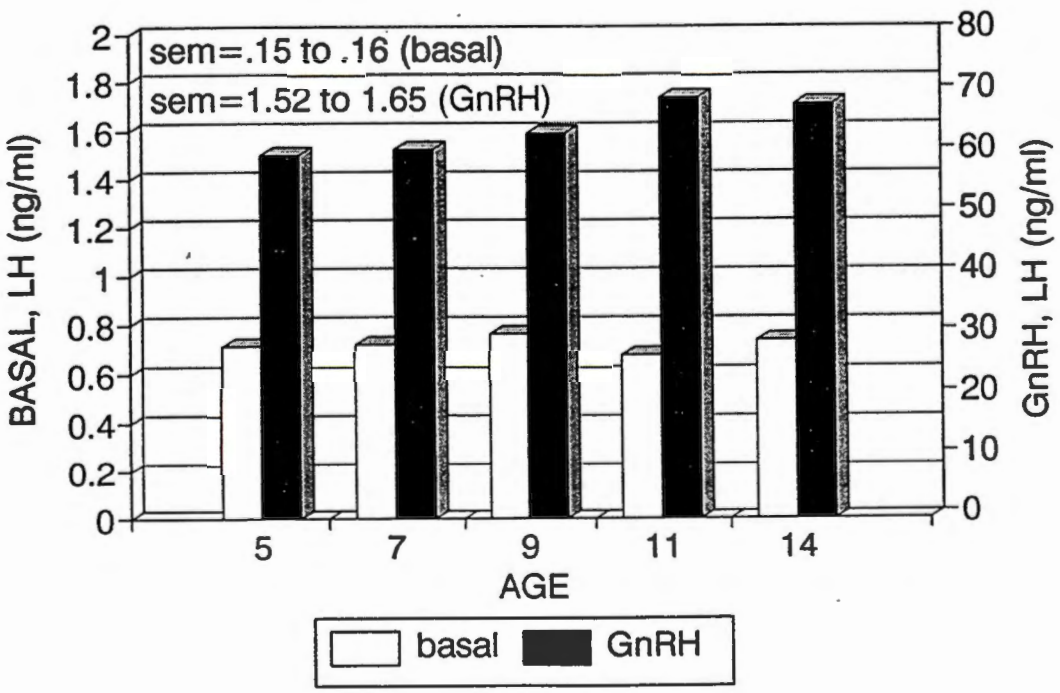

Fig 2 Age-related basal and GnRH-stimulated LH concentration in the beef heifer (5-14 mo).

Correlation of Gonadotrophin Concentrations with Number of Follicles in the 16 mo Ovary and Body Weight at Various Ages

FSH and Number of Primary, Growing, Normal Vesicular, Atretic Vesicular and Total Vesicular Follicles

No significant correlations were found at any age with either basal or GnRH stimulated secretion.

FSH and Body Weight

GnRH-stimulated secretion was positively correlated with weaning weight only at 5 mo and negatively correlated with yearling weight at 5 and 7 mo (Table 1). GnRH-stimulated secretion was negatively correlated with birth weight at 7 mo ($r = -.26$, $p = 0.07$). Basal concentration was negatively correlated with birth weight and yearling weight at 9, 11 and 14 mo, and weaning weight at 9 and 14 mo (Table 2). However, basal concentration was not correlated with body weight at 5 and 7 mo.

LH and Number of Primary, Growing, Normal Vesicular, Atretic Vesicular and Total Vesicular Follicles

Basal secretion was positively correlated with number of secondary follicles ($r = .25$, $p=0.07$) and number of primary follicles ($r = .32$, $p=0.02$) at 5 and 9 mo, respectively. Additionally, basal secretion was negatively correlated with number of atretic vesicular follicles ($r = -.26$, $p=0.06$) at 7 mo. No correlations were found with basal secretion at 11 and

Table 1. Correlations between GnRH-stimulated FSH secretion at various ages and birth, weaning and yearling weight.

		r/probability		
Age(mo)	Treatment	Birth wt	Weaning wt	Yearling wt
5	GnRH	NS[a]	.34/0.01	-.35/0.05
7	GnRH	-.26/0.07	NS	-.35/0.06
9	GnRH	NS	NS	NS
11	GnRH	NS	NS	NS
14	GnRH	NS	NS	NS

[a]No significant correlation was found

Table 2. Correlations between basal FSH concentrations at various ages and birth, weaning and yearling weight.

		r/probability		
Age(mo)	Treatment	Birth wt	Weaning wt	Yearling wt
5	Basal	NS[a]	NS	NS
7	Basal	NS	NS	NS
9	Basal	-.25/0.08	-.44/0.01	-.39/0.02
11	Basal	-.40/0.01	NS	-.41/0.01
14	Basal	-.26/0.06	-.37/0.01	-.48/0.01

[a]No significant correlation was found

14 mo, and GnRH-stimulated secretion at any age.

LH and Body Weight

GnRH-stimulated secretion was positively correlated with weaning weight at all ages except 9 mo, and yearling weight at all ages except 14 mo (Table 3). Basal secretion was only correlated with weaning weight at 7 and 14 mo (Table 4). No other correlations were found.

Correlation of Prepuberal Gonadotrophin concentration with number of Follicles in the 16 mo Ovary and Body Weight

One value for prepuberal gonadotrophin concentration (basal and GnRH-stimulated) was determined by taking the mean of 5, 7, 9 and 11 mo for each heifer. This value represented prepuberal gonadotrophin concentrations. Basal and GnRH-stimulated gonadotrophin secretion was then correlated with number of ovarian follicles and birth, weaning and yearling weight.

FSH and Number of Primary, Growing, Normal Vesicular, Atretic Vesicular and Total Vesicular Follicles

Similar to previous results, no significant correlations were found with either basal or GnRH-stimulated secretion in the prepuberal heifer.

Table 3. Correlations between GnRH-stimulated LH secretion at various ages and birth, weaning and yearling weight.

Age(mo)	Treatment	r/probability		
		Birth wt	Weaning wt	Yearling wt
5	GnRH	NS[a]	.32/0.02	.38/0.03
7	GnRH	NS	.23/0.10	.35/0.06
9	GnRH	NS	NS	.30/0.09
11	GnRH	NS	.31/0.02	.30/0.09
14	GnRH	NS	.36/0.01	NS

[a]No significant correlation was found

Table 4. Correlations between basal LH concentrations at various ages and birth, weaning and yearling weight.

Age(mo)	Treatment	r/probability		
		Birth wt	Weaning wt	Yearling wt
5	Basal	NS[a]	NS	NS
7	Basal	NS	.32/0.02	NS
9	Basal	NS	NS	NS
11	Basal	NS	NS	NS
14	Basal	NS	.49/0.001	NS

[a]No significant correlation was found

FSH and Body Weight

Basal concentration was not correlated with body weight at 5 and 7 mo; however, when ages were combined basal concentration was negatively correlated with birth weight ($r = -.32$, $p < 0.03$), weaning weight ($r = -.31$, $p < 0.03$), and yearling weight ($r = -.44$, $p < 0.02$). In contrast, GnRH-stimulated secretion in the previous results was significant at 5 and 7 mo; however, when combined with 9 and 11 mo no significant correlations were found with GnRH-stimulated secretion in the prepuberal heifer.

LH and Number of Primary, Growing, Normal Vesicular, Atretic Vesicular and Total Vesicular Follicles

In previous results basal concentration of LH was only correlated with number of primary follicles at 9 mo, but when ages (5,7,9 & 11) were combined correlation coefficient and significance decreased ($r = .26$, $p = 0.06$) as expected. No other correlations were found with either basal or GnRH-simulated LH secretion.

LH and Body Weight

Similar to previous results, weaning weight was positively correlated with both basal concentration ($r = .25$, $p = 0.07$) and GnRH-stimulated LH secretion ($r = .26$, $p = 0.05$). Additionally, yearling weight was positively correlated with GnRH-stimulated secretion ($r = .33$, $p = 0.06$). No other

correlations were found with either basal or GnRH-stimulated secretion.

Correlations Between Gonadotrophins

Stimulated FSH secretion was positively correlated with stimulated LH secretion at 5 ($r = .42$, $p < 0.01$), 7 ($r = .33$, $p < 0.02$), 9 ($r = .26$, $p = 0.07$), 11 ($r = .31$, $p < 0.03$) and 14 ($r = .27$, $p = 0.06$) mo. Basal FSH and LH secretion was not correlated at any age. However, basal and GnRH-stimulated FSH secretion was correlated at 7 ($r = .45$, $p < 0.01$), 9 ($r = .27$, $p = 0.06$) and 11 ($r = .24$, $p = 0.09$) mo, but not at 5 and 14 mo. Basal and GnRH-stimulated LH secretion was not correlated at any age.

Follicle Counts

Heifers were ranked from highest to lowest according to number of primary follicles (Table 5). Mean follicular numbers were: 98,593 (primary), 3,519 (secondary) 33 (normal vesicular) and 26 (atretic vesicular).

Correlations Between Classes of Follicles

Number of primary follicles was correlated with number of secondary ($r = .44$, $p < 0.01$), number of normal vesicular ($r =$

Table 5. Follicular numbers of fifty-two 16 mo old beef heifers.

			VESICULAR[c]		
ANO	PRIMARY[a] ($\times 10^3$)	SEC[b] ($\times 10^3$)	NORMAL[d]	ATRETIC[e]	TOTAL
412	395.8	1.9	29	17	46
420	308.1	8.4	47	47	94
5312	267.5	2.3	42	23	65
120	209.1	7.5	56	7	63
552	187.3	2.4	34	25	59
350	176.5	9.9	40	41	81
2080	174.0	3.9	32	15	47
5321	168.3	2.9	62	44	106
3412	166.4	8.7	40	32	62
610	163.7	5.3	37	49	86
250	159.9	6.2	54	38	92
3101	157.9	2.8	44	22	66
732	141.1	7.2	31	20	51
5171	141.1	1.9	63	31	94
310	138.4	5.4	29	25	54
2350	123.9	6.9	62	45	107
5341	119.9	4.7	31	16	47
60	117.9	6.3	34	27	61
2701	108.9	1.4	42	32	74
5222	100.4	2.8	45	53	98
5151	96.4	3.8	41	39	80
390	95.9	5.4	34	20	54
2351	92.9	3.4	64	36	100
3080	92.7	3.1	73	72	145
2562	89.7	4.5	20	49	69
2592	89.7	11.4	39	27	66
3451	84.7	1.6	57	20	77
4431	81.2	1.3	50	34	84
3152	76.9	4.9	40	39	79
2881	71.4	3.2	6	17	23
5252	67.3	1.6	17	11	28
5161	66.4	3.2	15	5	20
5211	58.9	1.7	33	30	63
2090	53.1	1.4	32	35	65
2391	52.8	1.6	15	13	28
450	51.2	3.1	41	44	85
650	50.1	2.6	21	17	38
2730	46.1	1.5	14	9	23
3841	36.9	3.0	23	19	42

[a]Oocyte with one layer of granulosa cells. [b]Oocyte that has two or more layers of granulosa cells. [c]Any follicle with an antrum. [d]Follicle with antrum and healthy granulosa cells. [e]Antral follicle with either necrotic or no granulosa cells.

Table 5. (Continued)

ANO	PRIMARY ($\times 10^3$)	SEC ($\times 10^3$)	VESICULAR		
			NORMAL	ATRETIC	TOTAL
4221	32.5	1.4	23	25	48
3530	30.0	1.1	3	0	3
480	28.8	3.1	24	40	64
700	27.7	3.0	28	24	52
3462	24.9	1.8	37	21	58
3750	23.2	4.9	36	35	71
5291	23.2	1.3	32	12	44
2612	22.1	2.0	8	3	11
5322	14.1	0.8	7	3	10
5221	9.8	0.6	11	5	16
2382	4.9	0.1	10	8	18
3571	2.6	0.6	9	4	13
230	2.2	1.1	2	2	4

.47, $p < 0.01$), number of atretic vesicular ($r=.28$, $p < 0.05$) and number of total vesicular ($r= .40$, $p < 0.01$) follicles. Number of secondary follicles was also correlated with number of normal vesicular ($r=.38$, $p < 0.01$), number of atretic vesicular ($r= .38$, $p < 0.01$) and number of total vesicular follicles ($r= .40$, $p < 0.01$).

Relationship of Gonadotrophin Concentrations (high, medium & low) at various ages to Number of Follicles in the 16 mo Ovary and Body Weight

Heifers were partitioned according to their basal concentration of each gonadotrophin and the response of each gonadotrophin to GnRH. At each age (5, 7, 9, 11 and 14 mo.) approximately 20% of the population was classified as either low or high with the remaining 60% being classified as medium (FSH:Table 6; LH:Table 7).

Follicle Stimulating Hormone

Differences between groups in their basal concentration and response to GnRH were significant at all ages ($p < 0.01$). The majority of the heifers did not change in classification as they aged. Fifty-four percent were stable in their basal classification and 68% were stable in their GnRH class. Twelve heifers changed basal groups three times (3X) and 12 heifers changed basal groups two times (2X) before the end of the study. Three heifers changed GnRH groups 3X and 14 heifers changed GnRH groups 2X in the course of the study.

Table 6. Distribution of heifers at various ages based on basal and GnRH-stimulated concentrations of FSH in serum[a]

Age (mo)	n[b]	Group	Treatment	FSH ng/ml
5	10	low	Basal	< 0.38
	11	high	Basal	> 0.53
	11	low	GnRH	< 2.76
	10	high	GnRH	> 4.28
7	11	low	Basal	< 0.37
	10	high	Basal	> 0.59
	11	low	GnRH	< 2.90
	10	high	GnRH	> 4.41
9	10	low	Basal	< 0.33
	10	high	Basal	> 0.53
	11	low	GnRH	< 3.20
	11	high	GnRH	> 4.48
11	10	low	Basal	< 0.34
	10	high	Basal	> 0.49
	13	low	GnRH	< 3.20
	12	high	GnRH	> 4.60
14	10	low	Basal	< 0.34
	11	high	Basal	> 0.49
	12	low	GnRH	< 3.12
	11	high	GnRH	> 4.20

[a] High and low groups contain the upper and lower 20% of the population, respectively. [b] All other heifers were included in "medium" group.

Table 7. Distribution of heifers at various ages based on basal and GnRH-stimulated concentrations of LH in serum[a]

Age (mo)	n[b]	Group	Treatment	LH ng/ml
5	10	low	Basal	< 0.51
	10	high	Basal	> 0.73
	11	low	GnRH	< 48.00
	10	high	GnRH	> 74.33
7	11	low	Basal	< 0.53
	10	high	Basal	> 0.76
	11	low	GnRH	< 51.85
	14	high	GnRH	> 75.00
9	11	low	Basal	< 0.57
	11	high	Basal	> 0.86
	10	low	GnRH	< 53.00
	12	high	GnRH	> 80.00
11	10	low	Basal	< 0.56
	12	high	Basal	> 0.81
	10	low	GnRH	< 53.00
	10	high	GnRH	> 86.00
14	10	low	Basal	< 0.57
	10	high	Basal	> 0.95
	10	low	GnRH	< 56.00
	10	high	GnRH	> 85.00

[a]High and low groups contain the upper and lower 20% of the population, respectively. [b] All other heifers were included in "medium" group.

With respect to FSH, groups did not differ in number of follicles of any class at any age ($p > 0.10$). At 5, 11 and 14 mo mean number of follicles (primary, secondary & vesicular) in the high GnRH group was numerically higher than the low group but the difference was not significant ($p > 0.10$). At 5 and 14 mo mean number of follicles increased with increasing basal FSH concentration but the difference was not significant ($p > 0.10$). No other trends were found.

No significant difference was found in body weight between basal or GnRH-stimulated groups at any age ($p > 0.10$). Additionally, there were no trends.

Correlations of Basal Concentrations of FSH and Follicle Numbers within Group:

Number of total vesicular follicles was negatively correlated with FSH at 14 mo in the high group ($r= -.54$, $p = 0.08$). Additionally, the low group at 14 mo was positively correlated with number of atretic vesicular follicles ($r= .59$, $p = 0.06$). No other correlations were found.

Correlations of GnRH-Stimulated Secretion of FSH and Follicle Numbers within Groups:

Number of normal vesicular follicles was positively correlated with FSH at 7 mo in the high group ($r= .55$, $p = 0.09$) and negatively correlated in the medium group ($r= -.44$, $p < 0.02$). No other correlations were found.

Correlations of Basal Concentrations of FSH and Body Weight within Groups:

Birth weight and FSH were negatively correlated at 9 ($r = -.61$, $p = 0.05$) and 11 ($r = -.71$, $p < 0.03$) mo in the high group. Furthermore, weaning weight was also negatively correlated with FSH at 11 ($r = -.75$, $p < 0.02$) and 14 ($r = -.67$, $p < 0.03$) mo in the high group. Yearling weight was negatively correlated with FSH at 9 ($r = -.74$, $p = 0.05$), 11 ($r = -.84$, $p < 0.01$) and 14 ($r = -.81$, $p < 0.03$) mo in the high group. No other correlations were found within groups.

Correlations of GnRH-Stimulated Secretion of FSH and Body Weight within Groups:

Birth weight was positively correlated at 7 mo in the medium group ($r = .48$, $p < 0.02$) and negatively correlated in the high group ($r = -.48$, $p = 0.08$). Additionally, birth weight was negatively correlated at 9 mo in the low group ($r = -.61$, $p < 0.04$). Weaning weight at 5 mo was positively correlated in the medium group ($r = .45$, $p = 0.06$) and negatively correlated in the high group ($r = -.60$, $p = 0.06$). Yearling weight was negatively correlated at 5 ($r = -.62$, $p = 0.07$) and 9 ($r = -.65$, $p = 0.05$) mo in the high group. Additionally, yearling weight was positively correlated at 7 mo in the low group ($r = .89$, $p < 0.05$). No other correlations were found.

Luteinizing Hormone

Differences between groups in their basal concentration and response to GnRH were significant at all ages ($p < 0.01$). In the majority of the heifers (59%), basal concentration classification changed with age. In contrast, 68% of the heifers did not change in GnRH classification with age. Fourteen heifers changed basal groups 3X and 17 heifers changed basal groups 2X before the end of the study. One heifer changed GnRH groups 3X and 16 heifers changed GnRH groups 2X before the end of the study.

Groups differed in number of primary follicles with basal concentration ($p = 0.05$; Fig 3) and GnRH-stimulated secretion ($p < 0.02$; Fig 4) at 14 mo. Additionally, GnRH stimulated groups differed in total number of vesicular follicles at 14 mo ($p = 0.09$; Fig 5). At 5, 9 and 11 mo mean follicle numbers (primary, secondary & vesicular) increased as basal LH concentration increased, however, this increase was not significant ($p > 0.10$). This trend did not exist at 7 mo. GnRH stimulated secretion also showed a similar positive increase in mean follicle numbers at all ages except 9 mo, however, they were not significant ($p > 0.10$).

GnRH-stimulated LH groups differed in their yearling weight at 5 ($p = 0.08$), 11 ($p < 0.01$; Table 8) and 14 ($p = 0.07$) mo. At 7 and 9 mo yearling weight increased from the low to high group, but the difference was not significant ($p > 0.10$). GnRH-stimulated LH groups showed a numerical

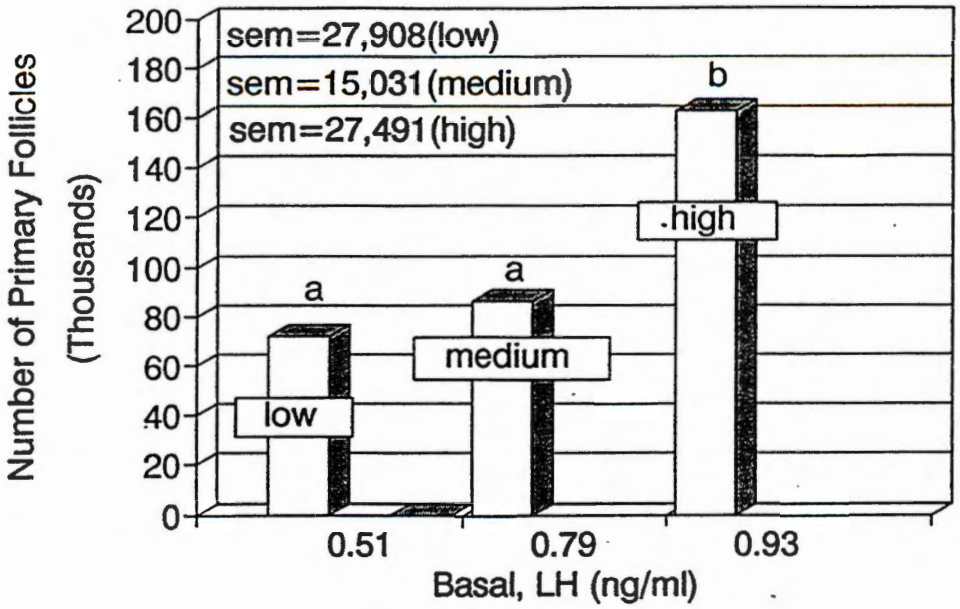

Fig 3 Mean number of primary follicles for low, medium and high LH basal concentration at 14 mo.

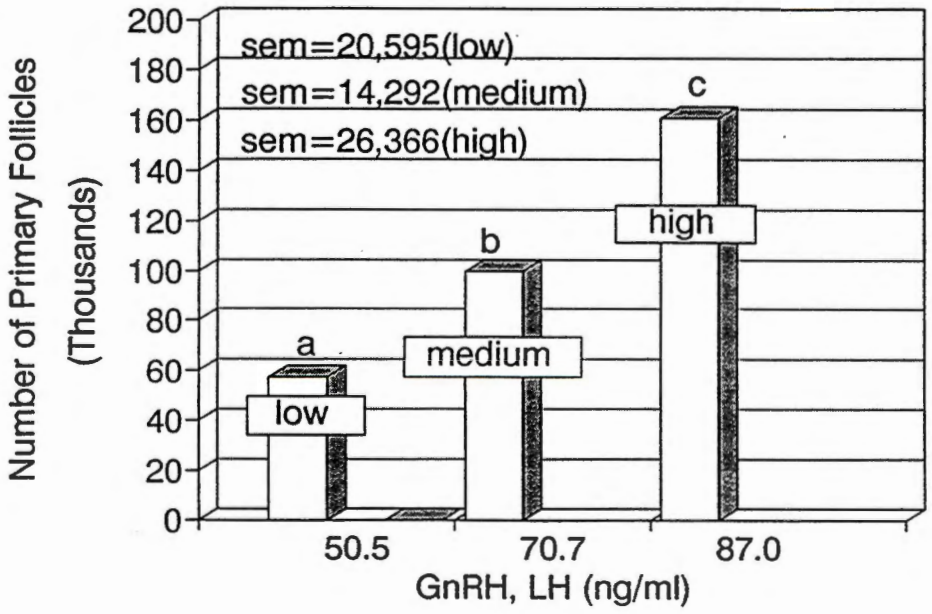

Fig 4 Mean number of primary follicles for low, medium and high LH GnRH-stimulated secretion at 14 mo.

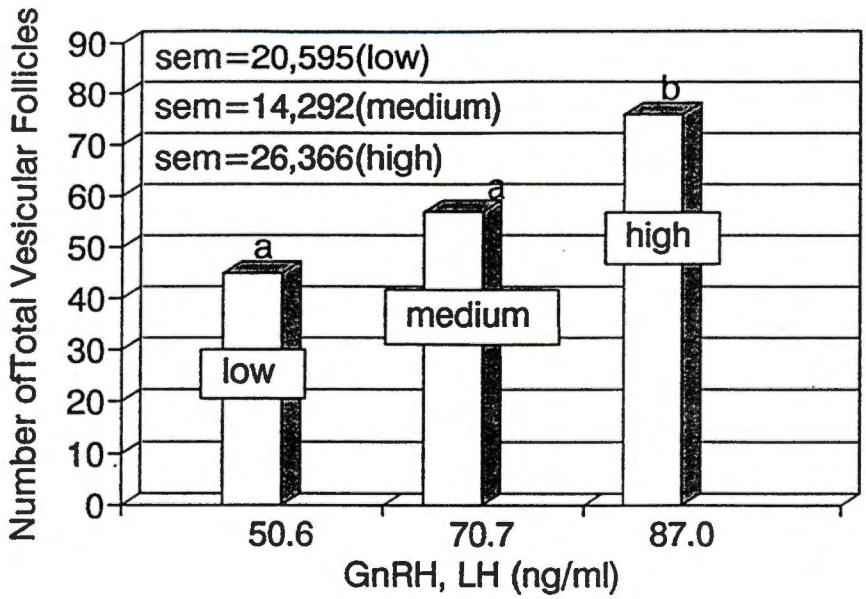

Fig 5 Mean number of total vesicular follicles for low, medium and high GnRH-stimulated LH secretion at 14 mo.

Table 8. Weight of GnRH-stimulated LH groups at 11 mo.

Group	Yearling Weight (Kg)
Low	219[a]
Medium	276[b]
High	280[b]

Means with unlike superscripts differ ($p < 0.01$).

increase from the low to high group in weaning weight at 5, 11 and 14 mo, but the difference was not significant ($p > 0.10$). No significant difference or trend was found with birth weight at any age.

Correlations of Basal Concentrations of LH and Number of Follicles within Groups:

Number of primary follicles was positively correlated with LH at 14 mo in the low ($r = .68$, $p < 0.03$) and high ($r = .56$, $p = 0.08$) groups. In contrast, number of secondary folliMwes was negatively correlated with basal at 7 mo ($r = -.40$, $p < 0.03$) in the medium group. However, at 14 mo the low group was positively correlated with number of secondary follicles ($r = .58$, $p = 0.07$). At 9 mo number of normal vesicular follicles was negatively correlated with LH in the medium group ($r = -.39$, $p < 0.05$). No other correlations were found.

Correlations of GnRH-Stimulated Secretion of LH and Follicle Numbers within Groups:

Number of secondary follicles was negatively correlated with GnRH-stimulated LH secretion at 14 mo in the high group ($r = -.64$, $p < 0.05$). Number of normal vesicular follicles was positively correlated at 5 mo in the high group ($r = .60$, $p = 0.06$) and at 7 mo in the medium group ($r = .33$, $p = 0.10$). Number of atretic vesicular follicles was positively correlated at 7 mo in the high group ($r = .56$, $p < 0.04$). No

other correlations were found within groups.

Correlations of Basal Concentrations of LH and Body Weight within Groups:

Birth weight was negatively correlated with LH at 7 mo in the high group ($r = -.63$, $p = 0.05$). Weaning weight was positively correlated at 5 mo in the high group ($r = .67$, $p < 0.04$) and at 11 mo in the medium group ($r = .48$, $p < 0.01$). Yearling weight was positively correlated with LH at 9 mo in the high group ($r = .71$, $p < 0.02$). No other significant correlations were found.

Correlations of GnRH-Stimulated LH Secretion and Body Weight within Groups:

Birth weight was positively correlated with LH at 7 mo in the medium group ($r = .48$, $p < 0.02$) and negatively correlated at 9 mo in the low group ($r = -.66$, $p < 0.04$). Weaning weight was positively correlated at 5 mo in the medium group ($r = .45$, $p = 0.06$) and negatively correlated in the high group ($r = -.60$, $p = 0.06$). Yearling weight was negatively correlated in the high group at 5 ($r = -.62$, $p = 0.07$) and 9 ($r = -.65$, $p = 0.05$) mo, however, it was positively correlated at 7 mo in the low group ($r = .89$, $p < 0.05$). No other correlations were found.

Relationship of Prepuberal Basal and GnRH Stimulated Gonadotrophin Concentrations to Number of Follicles in the 16 mo Ovary and Body Weight

The mean of heifers' basal concentrations and GnRH stimulated secretions were derived from 5, 7, 9 and 11 mo. Heifers were then partitioned according to basal concentrations of each gonadotrophin and the response of each gonadotrophin to GnRH. As before approximately 20% of the population was classified as either low or high responders with the remaining 60% being classified as medium responders (Table 9).

Follicle Stimulating Hormone

Correlations of Basal Concentrations of FSH and Follicle Numbers within Group:

In contrast to individual ages, no significant correlations were found between FSH and number of follicles.

Correlations of GnRH-Stimulated Secretion of FSH and Follicle Numbers within Group:

GnRH-stimulated secretion was positively correlated with number of normal vesicular follicles in the low group ($r = .57$, $p = 0.08$). In contrast to earlier results, no correlations were found in medium and high groups.

Table 9. Distribution of prepuberal heifers based on basal and GnRH-stimulated concentrations of gonadotrophins in serum[a]

Gonadotrophin	n[b]	Group	Treatment	ng/ml
FSH	11	low	Basal	< 0.38
	10	high	Basal	> 0.51
	10	low	GnRH	< 3.12
	11	high	GnRH	> 4.31
LH	10	low	Basal	< 0.60
	14	high	Basal	> 0.72
	10	low	GnRH	< 52.00
	11	high	GnRH	> 80.00

[a] High and low groups contain the upper and lower 20% of the population, respectively. [b] All other heifers were included in "medium" group.

Correlations of Basal Concentrations of FSH and Body Weight within Groups:

A similar negative correlation was found between basal concentration and weaning weight in the high group ($r = -.61$, $p < 0.06$). Additionally, weaning weight was positively correlated in the low group ($r = .61$, $p = 0.05$). In contrast, no significant correlation was found with birth and yearling weight within groups.

Correlations of GnRH-Stimulated FSH Secretion and Body Weight within Groups:

In contrast to individual age results, no significant correlations were found with GnRH stimulated FSH secretion and body weight when ages (5, 7, 9 & 11 mo) were combined.

Luteinizing Hormone

Correlations of Basal Concentrations of LH and Follicle Numbers within Groups:

In contrast to individual age results, number of primary follicles was positively correlated in the low group ($r = .67$, $p < 0.01$). Additionally, number of secondary follicles was positively correlated in the low group ($r = .59$, $p = 0.08$). No significant correlations were found in the medium group.

Correlations of GnRH-Stimulated Secretion of LH and Follicle Numbers within Group:

In contrast to individual age results, no significant correlations were found with GnRH stimulated secretion of LH and follicle numbers when ages (5, 7, 9 & 11 mo) were combined.

Correlations of Basal Concentrations of LH and Body Weight within Groups:

In contrast to individual age results, birth weight was positively correlated in the low group ($r = .74$, $p < 0.02$). And no significant correlations were found with weaning and yearling weight in the medium or high group.

Correlations of GnRH-Stimulated LH Secretion and Body Weight within Groups:

In contrast to individual age results, GnRH-stimulated LH secretion was negatively correlated with birth weight ($r = -.76$, $p < 0.01$) and yearling weight ($r = -.59$, $p = 0.05$) in the high group. And no significant correlations were found with birth, weaning and yearling weight in the low or medium groups.

DISCUSSION

Age Effects

The results show that the prepuberal gonadotrophin response to GnRH stimulation changes with age in the heifer. The FSH response increased significantly between 5 and 7 mo ($p < 0.01$) and then decreased at 14 mo ($p < 0.01$). This is in agreement with Dodson et al. (1988) who reported that mean FSH concentration in serum increased from 19 weeks to 35 weeks of age in the heifer. In contrast, McLeod et al. (1984) found no significant difference between 4 and 10 mo with 5 μg injection of GnRH. In the present study, at 9 mo the injection dosage was increased from 5 μg to 10 μg. The difference seen between McLeod et al. (1984) and the present study in GnRH stimulated concentration of FSH is due to the increased sensitivity of the pituitary to GnRH stimulation with increasing age. Data cited by Scham et al. (1981) suggested that age related concentrations of FSH tended to rise and fall in parallel with LH during calfhood (< 8 mo), but to a lesser extent, during the prepuberal period (> 8 mo). In our study, the FSH response to GnRH stimulation increased only until 9 mo whereas the LH response continued to rise (Fig 1 & 2). Basal FSH concentrations did not change significantly with age ($p > 0.10$), but did show a downward trend as age increased. Schillo et al. (1983) also reported that serum concentration

of FSH tended to decrease between 6 and 12 mo of age in the Crossbred (Angus X Holstein) heifer. This may be a result of increasing number of antral follicles (Erickson, 1967; Hansen et al. 1981) producing 17β-estradiol and inhibin.

Magnitude of the LH response to GnRH stimulation significantly increased with age: lowest at 5 mo, highest at 11 mo ($p < 0.01$) and unchanged at 14 mo (Fig 2). The response of LH to GnRH was 60 ng/ml at 5 mo and 68 ng/ml at 14 mo. LH in the peripheral circulation has been shown to increase with age in the prepuberal heifer (Dodson et al. 1988; Scham et al. 1981). In contrast, Barnes et al. (1980) reported that the GnRH stimulated release of LH was not different at 3, 6 and 9 mo. In the present study, stimulated LH secretion at 5 and 7 mo was not significantly different ($p > 0.10$; 60 ng/ml and 61 ng/ml, respectively), but 5 and 9 mo were significantly different ($p < 0.10$; 60 ng/ml and 64 ng/ml, respectively). The difference detected in the present study is likely due to the potent GnRH agonist used. The GnRH analog used is 50 times the potency of the native hormone (Chenault et al. 1990), and will essentially empty the pituitary of releasable LH. Barnes et al. (1980) used a GnRH (200 μg) of lesser potency and therefore, small age differences would probably not be detected.

In our study, basal concentration of LH did not change significantly between 5 and 14 mo (Fig 2). This agrees with findings by McLeod et al. (1984) and Kiser et al. (1981) who

reported that basal concentrations of LH were similar for heifers of 4 and 10 mo of age.

Ovarian Follicles

Number of primary follicles were positively correlated with number of secondary follicles ($r = .44$, $p < 0.01$), normal vesicular ($r = .47$, $p < 0.01$) and total vesicular follicles ($r = .41$, $p < 0.01$). Erickson (1967) found that ovaries from heifers containing more primary follicles had a greater number of vesicular follicles than ovaries containing a lower number of primary follicles. In general, as can be seen from Table 5, heifers with high primary follicle numbers had high numbers of secondary, normal vesicular, atretic vesicular and total vesicular follicle numbers. Data from Boni et al. (1993) suggest that number of vesicular follicle is a stable feature of a cow and since a positive association exists between number of primary and vesicular follicles, hormones should reflect number of follicles and follicles, to some degree, should reflect reproductive ability.

FSH and Number of Follicles

Basal and GnRH stimulated FSH secretion were not associated with number of primary, secondary, normal vesicular, atretic vesicular and total vesicular follicles in

the 16 mo ovary (pg 16). However, when the population was partitioned into low, medium and high groups associations between classes of follicles and concentration of FSH became significant in some cases. Significance was confined to 14 mo for basal concentration in the high and low group and 7 mo for GnRH-stimulated FSH secretion in the medium and high group (pg 27). Heifers were considered to be stable in their FSH response if they changed groups no more than once. Fifty-four percent were stable in their basal secretion of FSH and 68% were stable in their response to GnRH. Basal and GnRH stimulated groups were not significantly different in number of follicles at any age ($p > 0.10$). However, at 5, 11 and 14 mo numbers of all classes of follicles in the high FSH group was numerically higher than the low group (pg 27). Even though these differences were not significant, it does suggest that a high FSH response to GnRH could reflect a superior number of follicles.

FSH and Body Weight

An age related negative correlation of basal and GnRH stimulated FSH secretion with birth weight and yearling weight, and to a lesser extent with weaning weight (GnRH: Table 1; basal: Table 2). Body weight was inversely related to basal FSH concentration at 9, 11 and 14 mo (Table 2). Additionally, when low, medium and high groups were

established the inverse relationship was restricted to the high group. Therefore, from the 9 to 14 mo basal FSH concentration decreased with increasing body weight. This agrees with Schillo et al. (1983) who reported that serum FSH concentration decreased with age, but the decrease was not attributed to increasing weight of the heifer.

Prepuberal (mean of ages 5, 7, 9 & 11 mo) GnRH-stimulated FSH secretion was not correlated with body weight because of the little change seen with increasing age. However when low, medium and high groups were established an inverse association was found primarily with yearling weight and to a lesser extent with birth weight and weaning weight in the high group at 5, 7 and 9 mo of age. There were also isolated positive correlations at 5 ($r = .45$, $p = 0.06$) and 7 ($r = .48$, $p < 0.02$) mo with birth weight and weaning weight, respectively, in the medium group, and one negative correlation at 9 ($r = -.61$, $p < 0.04$) mo in the low group with birth weight. These appear to be random events and have no apparent meaning.

LH and Number of Follicles

Basal and GnRH stimulated LH secretion could be a useful indicators of reproductive capacity in the heifer. As seen from Figures 3, 4 and 5, number of follicles significantly increased from the low to high group ($p < 0.10$). This difference was only significant at 14 mo; however, a similar

trend existed at 5, 9 and 11 mo for basal concentration and 5, 7 and 11 mo for GnRH stimulated secretion. The reason the differences were not significant in the earlier ages is because heifers changed groups. Some heifers would change from (1) low to medium ,(2) low to high, (3) high to medium or (4) high to low. Heifers were most variable in their basal levels of LH with 59% of the population changing groups two or more times during the course of the study and least variable in their response to GnRH with 68% remaining in their starting group. However, the difference seen at 14 mo with basal and GnRH stimulated LH secretion could prove to be useful.

LH and Body Weight

GnRH stimulated LH secretion was positively associated with weaning weight and yearling weight at all ages except 9 and 14 mo, respectively. However, unlike that seen with basal FSH, basal LH was primarily not associated with body weight. A few isolated incidents of positive and negative correlations were noted, but these appeared to be random events with no apparent meaning. When partitioned into groups high GnRH responders at 5, 11 and 14 mo were heavier as yearlings ($p < 0.10$). And a similar trend was seen at 7 and 9 mo, but the differences were not significant ($p > 0.10$). Weaning weight increased from the low to the high group at 5, 11 and 14 mo, but the differences were not significant ($p > 0.10$). These

data suggest that heifers with greater yearling weights are superior in pituitary stores of LH. Therefore, given that mean ovarian follicles increase from the low to high group at 14 mo, selecting heifers by yearling body weight might be a useful means for identifying heifers of superior reproductive potential.

Conclusions

Basal concentration of FSH and LH do not change significantly in the prepuberal beef heifer between 5 and 14 months. In contrast, the prepuberal gonadotrophin response to GnRH stimulation changes with age. Unlike FSH, peripheral basal and GnRH stimulated secretion of LH was associated with number of follicles. Additionally, FSH concentrations were negatively associated with body weight and LH was positively associated. These data indicates that gonadotrophin concentrations in the peripheral blood along with body weight might be useful indicators of reproductive potential in the beef heifer. Heifers with heavier yearling weights and higher concentration of gonadotrophins (particularly LH) could be used as criteria for selecting replacement heifers.

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Study II

Effects of Successive Doses of GnRH on Gonadotrophin Release in the Angus Heifer

ABSTRACT

A study was conducted to determine the effects of successive doses of GnRH on the secretion of FSH and LH. Forty prepuberal Angus heifers were separated into two groups (n=20). The first group of 20 heifers was given two 10 μg subcutaneous injections of GnRH analog at a 1 day interval, and the second group was given two subcutaneous injections of GnRH analog at a 2 day interval. GnRH-stimulated gonadotrophin secretion was estimated from the total contained in the 1.5 and 3 hour bleedings following the injection of GnRH. Gonadotrophins were quantified by RIA.

Basal secretion of FSH was not affected at either one or two days after GnRH. Basal secretion of LH was reduced at the 1 day interval ($p < 0.001$), but had returned to normal two days after GnRH. At one day after the first dose of GnRH, the amount of FSH released by a second dose of GnRH was reduced to 49% of that released by the first dose ($p < 0.05$). When the interval between doses was increased to two days, the response to the second dose was still only 46% of that of the first dose. GnRH-stimulated secretion of LH was reduced by 83% at the 1 day interval, but the difference between doses was narrowed to 74% at the 2 day interval ($p < 0.01$).

These results suggest that (1) relative to LH, stores of FSH are small, (2) more than 2 days elapse before replacement of FSH stores is begun, (3) replacement of LH stores is in

progress 2 days after GNRH and (4) FSH secretion takes precedence over storage. Furthermore, the difference in the way these hormones respond to GnRH may help explain differences in the direction (+ or -) and degree to which they are correlated with follicular numbers and body weight.

INTRODUCTION

Effects of GnRH on FSH and LH secretion

The anterior pituitary houses specialized cells known as gonadotrophs that synthesize and secrete gonadotrophins (FSH and LH). In the rat it has been determined that gonadotrophs comprise about 15% of the cell population, and they can be either (1) multihormonal secreting both FSH and LH, or (2) momohormonal secreting either FSH or LH (Lloyd and Childs, 1988; Childs et al. 1982). Gonadotroph populations may change depending on the endocrine state, but are mainly comprised of the multihormonal type (Lloyd and Childs, 1988).

GnRH stimulates the synthesis and secretion of both gonadotrophins (Nett, 1990). Gonadotrophins are composed of two subunits, identical α-subunits and different β-subunits which determine biological activity (McCann and Ojeda, 1992). The genes coding for the two β-subunits are located on separate chromosomes and are likely to be differentially regulated (Pierce, 1988).

Though GnRH is important for the synthesis and secretion of FSH and LH, FSH is less dependent on GnRH than is LH (Price, 1991). Studies in the ewe utilizing techniques such as hypothalamic pituitary disconnection (Hamernik et al., 1986), passive immunization against GnRH (Sakurai et al., 1992), and GnRH antagonists (Brooks et al., 1993; Campbell et

al., 1990) which prevent GnRH from binding its receptor causes LH secretion to be almost undetectable, but has little effect on basal FSH concentration in the short-term (Kile and Nett, 1994). Data from the sheep (Brooks et al., 1993) suggests that FSH may be secreted as it is synthesized, suggesting that little FSH is stored by the gonadotroph. This hypothesis has not been tested in the bovine. Therefore, the objective of this study was to define differences in the secretion of the gonadotrophins in response to successive doses of GnRH in the Angus heifer.

MATERIALS AND METHODS

Experimental Design

Forty prepuberal Angus heifers with an average age of 10 months were used in this study. The animals were divided into two groups of twenty. The first group of 20 heifers was given two 10μg subcutaneous injections of Gonadotrophin Releasing Hormone (GnRH) analog (des-Gly^{10}, [$_D$-Ala^6]-LUTEINIZING HORMONE RELEASING HORMONE Ethylamide) at a 1 day interval, and the second group was given two subcutaneous injections of GnRH analog at a 2 day interval. Gonadotrophin levels were quantified via blood samples collected at 0, 1.5 and 3 hours after the injection of GnRH. Basal secretion was estimated from the zero bleeding and GnRH stimulated gonadotrophin secretion was estimated from the total contained in the 1.5 and 3 hour bleedings. Utilizing techniques described in study I, RIA's were used to quantify the gonadotrophins.

Statistical Analysis

Means and standard deviations were done by General Linear Model (GLM) by SAS.

RESULTS

Effects of successive doses of GnRH on basal secretion

No significant difference was found in basal secretion of FSH between intervals (Table 1). However, basal secretion of LH decreased at the 1-day interval ($p < 0.001$) but returned to normal at the 2-day interval (Table 2).

Effect of Successive Doses of GnRH on the Gonadotrophin Response

FSH

GnRH stimulated FSH secretion decreased from 4.1 $\pm$0.68 ng/ml to 2.1 $\pm$0.46 at the 1-day interval and from 3.9 $\pm$0.78 ng/ml to 1.8 $\pm$0.48 ng/ml at the 2-day interval ($p < 0.05$). Hence, GnRH stimulation of FSH secretion was reduced by 49% and 54% during the 1-day and 2-day interval, respectfully, but intervals were not significantly different ($p > 0.10$).

LH

GnRH stimulated LH secretion decreased from 71 $\pm$11 ng/ml to 12 $\pm$3 ng/ml at the 1 day interval but recovery was evident at the 2 day interval [72 $\pm$15 ng/ml (day 0) and 19 $\pm$6 ng/ml (day 2), ($p < 0.001$)]. An 83% difference existed at day 1, but the difference was reduced to 74% at day 2 ($p < 0.01$)

Table 1. FSH release in response to a GnRH challenge given at 1 or 2 days after an initial challenge in the prepuberal beef heifer.

		FSH ng/ml			
INTERVAL BETWEEN CHALLENGES	DAY	BASAL		GnRH	
1 DAY	0	0.44	±0.11	4.1	±0.68
	1	0.51	±0.12	2.1	±0.46
2 DAY	0	0.43	±0.12	3.9	±0.78
	2	0.46	±0.11	1.8	±0.48

Table 2. LH release in response to a GnRH challenge given at 1 or 2 days after an initial challenge in the prepuberal beef heifer.

		LH ng/ml			
INTERVAL BETWEEN CHALLENGES	DAY	BASAL		GnRH	
1 DAY	0	0.59	±0.12	71	±11
	1	0.41	±0.07	12	±3
2 DAY	0	0.59	±0.07	72	±15
	2	0.61	±0.16	19	±6

Thus, GnRH stimulated LH secretion at the 2-day interval was significantly higher than the 1-day interval ($p<0.001$).

DISCUSSION

The difference between the amount of FSH secreted at the first GnRH challenge (day 0) and that secreted at the second GnRH challenge given one day later was 51%. When the interval between the first and second GnRH challenge was increased to two days, the difference between the challenges was 46%. These results indicate that pituitary stores of FSH are not replaced immediately and that more than 2 days elapse before the process of recovery of FSH stores is begun. In contrast, LH release at the 2 day interval showed a significant increase over the 1 day interval: 17% 1-day interval, 26% 2-day interval. Gong et al. (1993) reported that the pituitary stores of LH will recover from GnRH in something less than 6 days.

Relative to LH, the amount of releasable or stored FSH is small (Tables 1 & 2). Ward et al. (1991) reported that the amount of extractable FSH in the bovine is 1.3 mg/kg of pituitary and LH is 60 mg/kg of pituitary. Therefore, the pituitary content of FSH is only 2% of that of LH. In the present study, mean FSH and LH secreted in response to the first dose of GnRH was 4.0 ng/ml and 71.5 ng/ml, respectively. Thus, releasable FSH was only 5% of the releasable LH. Absence of an effect of successive doses of GnRH on basal concentrations of FSH suggests that with FSH, secretion takes precedence over storage. It is possible that in addition to

a GnRH dependent pathway FSH is being secreted via a constitutive pathway. A constitutive pathway is defined as secretion which does not require a second messenger such as calcium for its release (Larson and Wise, 1994). Cells which use this pathway have little product in storage and increase and decrease synthesis to accommodate demand (Larson and Wise, 1994). Kyle and Nett (1994) suggested that FSH secretion, unlike LH, is not dependent upon increased intracellular calcium as a primary stimulus for inducing secretion. Larson and Wise (1994) reported that prolactin, a 23,000 molecular weight protein hormone (Norman and Litwack, 1987), is predominantly secreted via a constitutive pathway in the rat. The molecular weight of FSH is around 30,000, thus, it is possible that FSH can be secreted by a constitutive pathway.

The difference in the way these hormones are secreted is reflected in the way they are correlated with follicular numbers and body weight in study I. In general, basal and GnRH-stimulated secretion of FSH was not associated with number of follicles but was inversely related to body weight. In contrast, basal and GnRH-stimulated secretion of LH was positively related to number of follicles and body weight. Therefore, the possibility that FSH may be secreted, in part, via a constitutive pathway is further substantiated by its inverse relationship with body weight in the heifer.

Conclusions

Relative to LH, amount of releasable or stored FSH is small and FSH that is stored is not readily secreted in response to GnRH. Pituitary stores of FSH are not replaced immediately and more than 2 days elapse before the process of recovery of FSH stores is begun. Absence of an effect of successive doses of GnRH on basal concentration of FSH suggests that secretion takes precedence over storage or a constitutive pathway may be utilized for secretion.

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APPENDICES

APPENDIX A

Radioiodination Procedures

LH: Chloramine-T technique (Brown et al., 1983)
protein: LER 1072-2

Materials needed:

- Plastic 1.5 ml Microcentrifuge vial
- 0.5 M Phosphate buffer (pH 7.5) (PB)
- 0.05 M Phosphate buffer (pH 7.5) (PB)
- Chloramine-T (1 μg/μl in 0.05 M PB)
- Sodium Metabisulfite (2 μg/μl in 0.05 M PB)
- Bovine Serum Albumin (BSA)
- G-25-M Sephadex column

Proceed in a step-wise addition:

1. 5 μg LER 1072-2 in 25 μl 0.5 M PB
2. Add 500 μCi (5 μl) ^{125}I (Amersham Co.)
3. Add 15 μg of Chloramine-T (15 μl)
4. Mix gently, for 30 seconds
5. Add 60 μg of Sodium Metabisulfite (30 μl)
6. Add 100 μl of eluent buffer (0.05 M PB + 0.1% BSA)
7. Load onto a G-25-M Sephadex column for separation
8. Elute with 0.05 M PB + 0.1% BSA (1 ml at a time)

FSH: Iodogen Technique (Bolt and Rollins, 1983)
Protein: USDA bFSH I-2

Materials needed:

- 12 X 75 culture tube (Baxter)
- 0.5 M PB (pH 7.4)
- 0.25 M PB (pH 7.4)
- 0.05 M PB (pH 7.4)
- 0.5 M Sodium Diphosphate solution
- Sodium Metabisulfite (25 μl/ml in 0.25 M PB)
- Potassium Iodide (1 mg/ml in 0.5 M Sodium Diphosphate)
- BSA
- G-25-M Sephadex column

Plate the reaction vessel with a solution of iodogen in chloroform at a concentration of 4 μg/50 μl.

1. 5 mg in 5 ml; iodogen/chloroform
2. 80 μl of # 1 to 900 μl chloroform
3. 50 μl of # 2 is put in the reaction vessel and the chloroform evaporated with Nitrogen

Proceed in a step-wise addition:

1. 5 μg bFSH-BP3 in 100 μl 0.5 M PB
2. Add 400 μCi ^{125}I (Amersham Co.)

3. Vortex for 4 minutes (thorough, but gentle)
4. Add 200 µl Sodium Metabisulfite
5. Add 200 µl Potassium Iodide
6. Load on G-25-M Sephadex column for separation
7. Elute with 0.05 M PB + 0.1% BSA (1 ml at a time)

Column Preparation:

Columns PD-10 Sephadex G-25-M are used to separate LH and FSH radiolabelled compounds. Approximately 10 ml of the elution buffer (0.05 M Sodium Phosphate buffer + 0.1% BSA) is used to rinse the column prior to use. Buffer pH is 7.5 and for FSH is 7.4.

Isolation Technique:

Upon loading iodinated material onto the column, isolation of the labelled protein is accomplished through fractionating the eluted material in 1 ml fractions. Radioactivity is monitored using a portable Geiger counter. Radioactivity is eluted from the column in two peaks; the first peak is the protein fraction and the second peak is the free iodide. The labelled protein is eluted after approximately 4-5 fractions with the free iodide coming off the column at approximately 9-10 fractions. The labelled protein is eluded after approximately 11-13 fractions with the free iodide coming off the column at approximately 20-25 fractions.

APPENDIX B

Radioimmunoassay Techniques

B-1. Protocol for LH RIA. Modified Brown (1983).

TUBE	TUBE#	RIA BUFFER (µl)	SAMPLE (µl)	1st Ab (µl)	TRACER (µl)
TC	1-3	----	----	----	100
NSB	4-6	600	----	----	100
B0	7-9	400	----	100	100
STANDARDS	10-30	200	200	100	100
QC	31-32	150	250	100	100
SAMPLES	33-	150	250**	100	100

**Sample volume (sample & buffer) must equal 400 µl. Use 100 µl sample and 300 µl buffer.

B-1 (continued) After 48 hour incubation add:

TUBE	TUBE#	NRS (µl)	2nd Ab (µl)	PEG (µl)
TC	1-3	----	----	----
NSB	4-6	100	100	500
BO	7-9	100	100	500
STANDARDS	10-30	100	100	500
QC	31-32	100	100	500
SAMPLE	33-	100	100	500

B-2 Protocol for FSH RIA. Modified Bolt & Rollins (1989).

TUBE	TUBE#	RIA BUFFER (µl)	SAMPLE (µl)	1st Ab (µl)	TRACER** (µl)
TC	1-3	----	----	----	100
NSB	4-6	700	----	----	100
B0	7-9	500	----	200	100
STANDARDS	10-33	300	200	200	100
QC	34-35	300	200	200	100
SAMPLES	36-	300	200	200	100

**Incubated at room temperature for 24 hours before adding tracer.

B-2 (continued) After 24 hours incubation with tracer add:

TUBE	TUBE#	NRS (μl)	2nd Ab (μl)	PEG (μl)
TC	1-3	----	----	----
NSB	4-6	100	100	500
B0	7-9	100	100	500
STANDARDS	10-33	100	100	500
QC	34-35	100	100	500
SAMPLES	36-	100	100	500

TC= Total count or the total amount (counts per minute) of tracer added to each tube.

NSB= Nonspecific binding, amount of interference of impurities in the assay tubes, buffer, etc.

B0= Total binding (%) capacity of the working dilution of tracer and antibody to be used as the basis for determining hormone concentrations.

STANDARDS= Known amounts of hormone used to construct the standard reference curve.

QC= Quality control, a standard unknown plasma sample used in assay to control intra- and interassay variation.

SAMPLES= The unknown amount of the hormone that is to be measured.

LH procedure:

Proceed in step-wise addition: (wear gloves at all times)

1. Add 200 μl standards to numbered assay tubes.
2. Add appropriate volume of plasma (μl) to tube.
3. Add correct volume (μl) of buffer to all tubes.
4. Add 200 μl of 1st Ab to all tubes except TC & NSB.
5. Add 100 μl of tracer to all tubes.
6. Vortex all tubes except TC.
7. Incubate 48 hours at room temperature.
8. Add 100 μl Normal Rabbit Serum (NRS) to all tubes except TC.
9. Add 100 μl of 2nd Ab to all tubes except TC.
10. Add 500 μl of Polyethylene glycol (PEG) to all tubes except TC.
11. Vortex all tubes except TC.
12. Incubate 5 hours at room temperature.
13. Centrifuge at 3000 rpm for 15 minutes.

14. Decant the supernatant into "Radioactive" labelled bottles.
15. Let tubes drain upside down for 10 minutes in box labelled "Radioactive" lined with foil and kimwipes.
16. Count the tubes in a gamma counter.
17. Discard gloves, foil, and kimwipes in a barrel labelled "Radioactive < 90 days".

FSH Procedure:

Proceed in step-wise addition: (wear gloves at all times)

1. Add 200 μl of standards to numbered assay tubes.
2. Add appropriate volume of plasma (μl) to tubes.
3. Add correct volume (μl) of buffer to all tubes.
4. Add 200 μl of 1st Ab to all tubes except TC & NSB.
5. Vortex all tubes except TC.
6. Incubate 24 hours at room temperature.
7. Add 100 μl tracer to all tubes.
8. Vortex all tubes except TC.
9. Incubate 24 hours at room temperature.
10. Continue with steps 8-17 in LH procedure.

APPENDIX C

Breed and Body Weight

C-1. Body weights and breeds of 52 ovariectomized heifers.

Cowid	Breed[a]	Birth weight	Weaning weight[b]	Yearling weight
0412	PH	75	471	614
0552	PH	77	505	624
0732	AN	85	534	608
2382	AN	52	515	626
2562	AN	66	486	666
2592	AN	86	512	728
2612	AN	47	316	326
3152	AN	61	500	622
3412	AN	48	468	482
3462	AN	73	438	484
5222	AN X PH	80	500	650
5252	(GV X AN) X AN	65	469	552
5312	AN X GRADE	85	583	682
5322	(GV X AN) X AN	70	529	596
2351	AN	59	442	544
2391	AN	70	515	626
2701	AN	71	504	594
2881	AN	60	515	650
3101	AN	74	429	508
3451	AN	57	502	552
3571	AN	59	442	504
3841	AN	70	537	570
4221	AN	65	551	542
4431	AN	72	474	496
5151	PH X AN	78	570	684
5161	PH X AN	90	553	706
5171	PH X AN	85	538	660
5211	PH X AN	70	517	640
5221	PH X AN	55	469	564
5291	PH X AN	90	541	670
5321	PH X AN	78	586	672
5341	PH X AN	70	539	593
0060	AN	68	433	N/A[c]
0120	AN	64	464	N/A
0230	AN	76	447	N/A
0250	AN	62	476	N/A
0310	AN	75	372	N/A

[a]Angus (AN); Polled Hereford (PH); Gelvieh (GV).
[b]Adjusted 205 day weaning weight was used.
[c]Yearling weight not available.

C-1. (Continued)

Cowid	Breed	Birth weight	Weaning weight	Yearling weight
0350	AN	73	428	N/A
0390	AN	78	558	N/A
0420	AN	71	430	N/A
0450	AN	75	378	N/A
0480	AN	82	488	N/A
0610	AN	71	424	N/A
0650	AN	60	468	N/A
0700	AN	76	455	N/A
2080	AN	86	455	N/A
2090	AN	73	419	N/A
2350	AN	78	357	N/A
2730	AN	75	448	N/A
3080	AN	77	467	N/A
3530	AN	88	455	N/A
3750	AN	54	493	N/A

VITA

Michael S. Rowland was born in Tazewell, TN. and received his high school education in Claiborne County. In 1991, he graduated with a Bachelor of Science degree in Animal Science from The University of Tennessee, Knoxville.

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