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Utilization of hormonal profiles as indicators of reproductive capacity in beef heifers

Patricia K. Hillyer

To the Graduate Council:

I am submitting herewith a thesis written by Patricia K. Hillyer entitled "Utilization of hormonal profiles as indicators of reproductive capacity in beef heifers." 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. Godkin, J.D. Smalling

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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

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John D. Snalling

Accepted for the Council:

Associate Vice Chancellor
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Date August 20, 1992

UTILIZATION OF HORMONAL PROFILES AS INDICATORS OF REPRODUCTIVE CAPACITY IN BEEF HEIFERS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Patricia K. Hillyer

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ABSTRACT

Two studies were conducted to examine the extent to which prepuberal hormone secretion can be used as an indicator of reproductive capacity in the beef heifer. The first involved prepuberal hormonal differences in 3 breeds of heifers (Angus, Crossbreds (SPxAN and HPxAN) and Polled Herefords). Basal and GnRH stimulated blood samples were obtained from Angus (AN, n=29), Crossbred (XB, n=14) and Polled Hereford (HP, n=14) at 6 ages (3, 5, 7, 9, 11 and 12 mo). Basal values resulted from the avg of 3 hourly bleedings; GnRH values resulted from the avg of bleeding effected 1.5 & 3 hours after GnRH. RIA's were conducted for luteinizing hormone (LH, ng/ml), follicle stimulating hormone (FSH, ng/ml), progesterone (P_4, pg/ml), androstenedione (Δ_4A, pg/ml), testosterone (T, pg/ml) and estradiol (E_2, pg/ml).

Polled Herefords were distinctive in having the highest levels of the gonadotrophins, the least amount of E_2 and a longer time to reach puberty. Crossbreds were significantly heavier at all ages and highest in their basal & GnRH stimulated secretion of T (at all ages) and Δ_4A (9, 11 & 12 mo). Angus showed a higher secretion of basal & GnRH stimulated secretion of Δ_4A at 3, 5 & 7 mo.

Ovulation (P_4 > 1 ng/ml) was observed in XB at 9 mo, AN at 11 mo and only 1 HP expressed high P_4 values by 12 mo of age. Groups of heifers that ovulated at an early age had

higher basal & GnRH simulated secretion of E_2 & Δ_4A at 3 & 9 mo respectively.

The second study was conducted over 2 years to examine the relationship between Δ_4A and gonadal steroids and their correlation with number of ovarian follicles. Ovaries were removed from 16 mo old AN heifers (n=20) for Year-1 and 14 mo old ANG (n=10) and XB heifers (n=8) for Year-2. Follicles were enumerated, classified and correlated with basal and GnRH stimulated steroid concentrations in serum. Heifers were bled at 3, 5, 7, 9, 11 & 12 mo of age. Heifers with the highest number of primary follicles had significantly higher Δ_4A at 9 & 11 mo and higher testosterone at 7 mo. Primary follicle numbers were positively correlated with secondary, growing, atretic and total vesicular follicles (r=.5).

These results suggest: 1) that Δ_4A, T and E_2 could be used to predict early age at puberty and 2) that basal and GnRH stimulated Δ_4A and T either before or around the time of weaning could be used to predict primary follicle numbers. Utilizing these results, prepuberal androgens in conjunction with E_2 could be used to predict the potential reproductive capacity of the beef heifer.

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INTRODUCTION

A major goal of the beef producer is to increase reproductive efficiency. Numerous studies have been conducted to examine the effects of heterosis, or hybrid vigor, on various crosses of cattle. All results indicated Crossbreds exceed Angus and Polled Herefords (with Angus exceeding the Polled Herefords) in lifetime capacity to reproduce (Cundiff, 1970). Pregnancy rates (Reynolds et al., 1986), number of calves weaned (Sacco et al., 1989; Peacock et al., 1971; Neumann, 1977) and rate of growth (Cundiff, 1970; Bailey et al., 1988) are also increased. Relationship of hormone secretion to these results have, as yet, not been examined.

As beef cattle researchers, it is our responsibility to develop techniques to assist the producer in attaining this goal. If we are able to predict the reproductive capacity of the beef heifer through the utilization of prepuberal hormone secretion, significant savings to the producer would result.

Two studies were conducted to evaluate whether such a test of reproductive capacity has merit. In the first study the extent to which three breeds of heifers differ in their prepuberal hormonal secretions was examined and in the second study, correlations between serum concentrations of gonadal steroids and follicular numbers were determined.

STUDY I

INTERBREED DIFFERENCES IN HORMONAL SECRETION IN PREPUBERAL BEEF HEIFERS

ABSTRACT

To examine the extent to which breeds differ in age at puberty and their capacity to secrete some of the reproductive hormones, the hormonal profiles of prepuberal Angus (AN, n=29), Crossbred (XB, n=14, SPxAN, HPxAN) and Polled Hereford (HP, n=14) heifers were evaluated at six ages (3, 5, 7, 9, 11 & 12 mo). Basal and GnRH-stimulated secretions were quantified. Basal values resulted from the average of 3 hourly bleedings & the GnRH response was assessed from the average of bleedings effected at 1.5 and 3 hours after the injection of GnRH. Radioimmunoassays were conducted for LH (ng/ml), FSH (ng/ml), P_4 (pg/ml), Δ_4A (pg/ml), T (pg/ml) and E_2 (pg/ml).

Polled Herefords were distinctive in having numerically higher gonadotropin values at 3, 5, 7 & 12 mo and lower E_2 values at 5 & 12 mo. Crossbreds had significantly higher basal and GnRH stimulated secretion of testosterone (T) at all ages examined. Angus were characterized by notably higher basal and GnRH augmented secretion of Δ_4A at 3, 5 and 7 mo of age. Forty-three percent of the Crossbred heifers had ovulated (P_4 > 1 ng/ml) at 9 mo of age, 44% of the AN at 11 mo and only one HP had ovulated by 12 mo. Early ovulators had higher basal and GnRH stimulated secretion of E_2 at 3 mo ($p<0.001$) and Δ_4A at 9 ($p<0.001$) and 11 mo ($p<0.001$).

These results suggest that basal and GnRH stimulated

secretion of Δ_4A, T & E_2 may signal an early age at puberty and that a high level of T may be one factor that accounts for the superior performance of the XB heifer.

INTRODUCTION

Heterosis:

Heterosis, or hybrid vigor, is defined by Van Vleck et al. (1987) as the extent to which crossbred progeny exceed the average performance of the parental lines.

Crossbred heifers have been reported to exceed both Angus and Polled Herefords in their lifetime capacity to reproduce, with Angus having an advantage over Polled Herefords (Cundiff, 1970; McCarter et al., 1991). In 1977, Neumann reported that 31% of Crossbreds were still in the herd after 11 to 14 years, versus only 16% of the Straightbreds. Reynolds et al. (1986) reported that pregnancy rates for Hereford x Angus (HPxAN) crosses were 5.2% higher than straightbred Hereford or Angus. They also reported that productivity (kg of calf/cow) of Herefords increased 16% and Angus 8.5% when bred to bulls of another breed. Performance of Crossbreds was superior to that of straightbreds in other studies (Peacock et al., 1971; Neumann, 1977; Reynolds et al., 1986; Bailey et al., 1988; Williams et al., 1991). In two separate studies, puberty in the heifer was associated with body weight. In the study of Cundiff (1970), weights of heifers that had attained puberty exceeded those that had not by 21.6 kg. The difference noted by Bailey & associates (1988) was 31.9 kg. First heat was exhibited anywhere from 35-40 days earlier in Crossbreds than straightbreds (Cundiff, 1970). The cause of this earlier age

at puberty for Crossbreds has not yet been investigated, but the difference in rate of maturity could be because of differences in reproductive hormone secretion.

Prepuberal Development:

Kinder and associates (1987) defined puberty as "the first behavioral oestrus accompanied by the development of a corpus luteum that is maintained for a period characteristic of a particular species." Age at puberty varies among breeds of cattle and ranges from 8-18 mo with an average of 12 mo (Blakely and Bade, 1990).

In heifers, first ovulation is triggered when the hypothalmo-pituitary axis loses its high sensitivity to the negative effects of 17β-estradiol, allowing the preovulatory LH surge to occur (Kinder et al., 1987). First ovulation is not entirely synonymous with puberty, since many heifers will have one to two "silent" ovulations before their first estrous (Kinder et al., 1987; Moran et al., 1989).

Follicle stimulating hormone promotes follicular growth by means of its effects on the granulosal cells. These cells cause the formation of the theca interna which in turn produces androgens. The androgens (Δ_4A & T) can then be aromatized to 17β-estradiol (E_2) (Anisworth et al., 1990). As the prepuberal concentration of E_2 rises, its negative effects decrease as a possible consequence of a reduction in the number of it's receptors in the anterior and medial basal

hypothalamus (Day et al., 1987; Kinder et al., 1987). This decrease in E_2 receptors is coincident with an increase in LH secretion. When pulses of LH reach one pulse/hour, ovulation can occur (Kinder et al., 1987).

Ovulation can be induced in the heifer long before puberty, since exogenous E_2 can induce an LH surge in the 7 mo old heifer and PMSG can cause ovulation in the calf (Howe et al., 1962; Kinder et al., 1987). In heifers, levels of LH and FSH increase from birth to 4 mo then diminish before increasing at puberty (Gonzalez-Padilla et al., 1975; Moran et al., 1989). Prior to puberty, two distinct elevations in progesterone occur and last anywhere from 2-5 days. The first occurs 11-18 days prior to puberty with an LH rise immediately following (called P_4 primed LH rise), while the second is observed between the primed LH increase and the actual LH surge (Gonzalez-Padilla et al., 1975). This increase in progesterone in heifers is from the ovary (Berardinelli et al., 1979), while the rise in P_4 prior to ovulation exhibited in rodents is of adrenal origin (Putnam et al., 1991). Estradiol has been reported to remain relatively low & constant until the approach of ovulation (Moran et al., 1989).

Factors Affecting Age at First

Ovulation and Puberty:

1. Weight: In a 2 year study by Arije and Wiltbank (1971), it was reported that Hereford heifers reached puberty at a breed dependent weight (249-254 kg) rather than at a certain age. Wiltbank and associates (1966) reported a mean weight of 251 kg for AN heifers and 247-282 kg for XB heifers (HHxAN, HHxSP, SPxAN; HH=Hereford, AN=Angus, SP=Shorthorn).

2. Nutrition: Imakawa et al. (1986) showed that cycling heifers whose nutrition was severely restricted became anestrus, even though they far exceeded the "critical" puberal body weight. Bushmich and associates (1980) reported increased propionate and volatile fatty acids was associated with increased ovarian weight. Meinert and associates (1992) reported that the feeding of Monensin (an ionophor) was associated with a reduced age at puberty without affecting body weight or composition, however, the mechanism of action is not fully understood.

3. Breed: Crossbred heifers reach puberty at an earlier age and lighter weight than straight-bred heifers (Cundiff, 1970; Bailey et al., 1988; also see pages 5 & 6).

4. Season: Studies have indicated that seasonal factors influence age at puberty in heifers. Schillo and associates (1983) performed a study in spring and fall born heifers where the second 6 mo of life was in a controlled environment giving a spring to autumn or autumn to spring sequence. Age at

puberty was influenced by date of birth, indicating natural environmental conditions to which heifers were exposed during the first 6 mo of life influenced age at puberty. Heifers born in the autumn tended to reach puberty earlier in life than heifers born in the spring.

Objectives:

Objectives of this study were to (1) obtain hormonal profiles of 3 breeds of puberal beef heifers (Angus, Crossbred and Polled Herefords), (2) investigate possible breed differences in basal and GnRH induced hormonal secretions and (3) determine whether prepuberal hormone secretion is significantly correlated with age at first ovulation.

MATERIALS AND METHODS

Experimental Design:

Twenty-nine Angus, 14 Polled Hereford and 14 Crossbred heifers were used in this study. Crossbreds (F1 crosses) were a mixture of Polled Hereford x Angus (HPxAN) and Shorthorn x Angus (SPxAN). The dams of the Crossbred calves were pure-bred Angus cows. This minimized milk production of the dam as a variable. The Polled Herefords used were part of a herd characterized by poor reproductive performance distinguished mainly by an early age at culling for reproductive reasons.

After weaning at 7 mo, all heifers were supplemented with hay (2 times/week) and silage (3 times/week) until 13 mo of age. They were allowed to drink and graze the pasture (fescue endophyte infected) ad libitum. Birth, weaning and yearling weights were recorded for all heifers.

Four developmental stages were studied: an early follicular stage (3 through 5 mo postnatal) marked by the first significant follicular growth and wide interanimal variability in LH and FSH secretion (Cato, 1986; Erickson, 1966); a late juvenile stage (6 through 8 mo) distinguished by a hypothalamus that has attained the ability to respond positively to an E_2 challenge (Staigmiller et al., 1979); a peripuberal stage (9 through 11 mo) where the hypothalamus is responsive to E_2 in all heifers and where some may have ovulated; and a puberal to postpuberal stage (12 through 14

mo) where almost all heifers have usually ovulated.

When the heifers were 3, 5, 7, 9, 11 and 12 mo of age, basal hormonal concentrations of serum were determined from the average of 3 bleedings effected at an hourly interval. Polled Herefords were not bled at 9 and 11 mo of age.

To determine the response of the pituitary and the ovary to a hormonal challenge, all heifers received a 5 μg intramuscular injection of the Gonadotropin Releasing Hormone (GnRH) analog (des Gly^{10}, [D-ala^{6}]-GnRH ethylamide), when the average ages were 3, 5 and 7 mo. At 9 mo the dose of GnRH was increased to 10 μg. Quantities of hormones secreted after GnRH are the average of blood samples collected 1.5 and 3 h after injection.

All blood samples were collected via the non-catheterized jugular vein. Serum separator collection tubes were place on ice immediately after bleeding & were centrifuged at 1764 x g for 20 minutes 3 hrs later. Serum was poured off and stored at -20°C until assayed (RIA) for its content of the gonadotropins (LH & FSH) and the steroids, progesterone, androstenedione, testosterone and estradiol (P_4, Δ_4A, T & E_2 respectively).

Radioiodination Procedures

The Cloramine-T method described by Brown et al. (1983) was used to radioiodinate luteinizing hormone (LH). Iodination and reference grades of LH were provided by Dr. L.

H. Reichert, Albany Medical School, New York.

Radioiodination of Follicle Stimulating Hormone (FSH) followed a modified version of the Iodogen method described by Bolt and Rollins (1983). Iodination and reference grade hormone were provided by Dr. Bolt, USDA, Beltsville, Maryland.

Protocols for LH and FSH radioiodination procedures are detailed in Appendix B.

Radioimmunoassay Techniques

Hormonal serum concentrations were determined by comparison of the unknown to the standard curve for all assays conducted (LH, FSH, P_4, Δ_4A, T and E_2).

Luteinizing hormone concentrations were measured using the method described by Brown and associates (1983). A reference curve (0.31 to 4 ng) was constructed using triplicate samples of standards. Antibody to LH (#15 antiovine LH) was provided by Dr. Gordon Niswender, Colorado State University. The antibody did not significantly cross-react with FSH, TSH or growth hormone (Niswender et al., 1969). Intra- and interassay variation was determined by measures of the LH content of pooled plasma in each assay. The intraassay coefficient of variation was <5.0 % while interassay variation was <7.0 %. Appendix C contains details of the LH assay.

Plasma FSH was measured utilizing a modified version of Bolt and Rollins technique (1983). A reference curve (.25 to

16 ng) was constructed using triplicate samples of standards. Follicle Stimulating Hormone antibody (USDA bFSH-BP3) was provided by Dr. D. J. Bolt of USDA. Bovine TSH had a cross-reactivity of 0.5% with this Ab, while bovine LH, Prolactin and Growth Hormone had a cross-reactivity of <0.09% (Bolt and Rollins, 1983). Intra- and interassay variation was determined as previously described for LH. The intraassay coefficient of variation was <5.0 % with interassay variability being <9.0%. Details of FSH procedures can be found in Appendix C.

Progesterone was measured utilizing modified RIA procedures described by Jackson et al. (1989). A reference curve (10 to 1000 pg) was constructed using duplicate samples of the standards. Progesterone antibody (#337-Ali Surve) was supplied by Dr. Ali H. Surve, Sandoz Research Institute, Hanover, New Jersey. Intra-assay coefficient of variation was < 3.4 % while inter-assay variation was <10%. Appendix C contains the details of the progesterone assay.

Androstenedione followed a modified RIA procedures described by Jackson and associates (1989). A reference curve (2.5 to 250 pg) was obtained using duplicate standard samples. Androstenedione antibody (X-322 Rao) was supplied by Dr. Pemmaraju N. Rao, Southwest Foundation for Biomedical Research, San Antonio, TX. Cross-reactivity of this Ab was reported as being 2.0 % with DHT, 5.0 % with DHEA and 0.8%< with other steroids (Babalola and Shapiro, 1988). Intra- and

inter-assay coefficient of variation were <5% and <7.9% respectively. Assay techniques for Δ_4A can be found in Appendix C.

Radioimmunoassays for testosterone followed a modified version of Jackson et al.(1989). A reference curve (2.5 to 250 pg) was obtained using duplicate standard samples. Testosterone Ab (Nis) was provided by Dr. G. D. Niswender, Colorado State University. This antibody's cross-reactivity showed 1% with 3α-androstanediol, <1% with Δ_4A and 1% with 3β-androstandediol (Amann and Walker, 1983). Intra-assay variation was <5%, while inter-assay was <10%. Detail of the testosterone assay can be found in Appendix C.

Estradiol (E_2) was measured using RIA protocol developed by Dr. Jack Britt (1990). A reference curve (0.15 to 10 pg) was constructed using triplicate samples of the standards. Estradiol antibody (Mason) was supplied by Dr. Norman Mason, Lilly Research Laboratories, Indianapolis, IN. Cross-reactivity was 0.9% with estrone and 2.2% with estradiol-17α (Kesler et al., 1977). Intra-assay coefficient of variation was < 5.9 % while inter-assay was < 10%. Details of the Estrogen assay can be found in Appendix C.

Recipes for all buffers and reagents are located in Appendix A.

Statistical Analysis

Least square means, standard deviations and standard errors were computed using General Linear Mixed Model (GLMM) (Blouin and Saxton, 1990) with a broad inference space. Utilizing the cell-mean model rather than the over-parameterized model, our model statement was as follows:

$$y_{ijkl} = age*trt*breed + animal + e_{ijkl}$$

where:

i = number of ages (1...6)

j = number of treatments (1, 2)

k = number of breeds (1, 2, 3)

l = number of animals (1...56)

RESULTS

Crossbred heifers (Table 1) were significantly heavier than the other two breeds at birth, weaning and as yearlings ($p<0.01$) with no significant difference between Angus and Polled Hereford.

Table 1. Birth, weaning and yearling weights of Polled Hereford, Crossbred and Angus heifers

Breed	Birth (kg)	Weaning (kg)	Yearling (kg)
AN	30[a]	215[a]	269[a]
HP	32[a]	215[a]	266[a]
XB	37[b]	250[b]	312[b]

Means in each column with unlike superscripts differ ($p<0.01$)

FOLLICLE STIMULATING HORMONE

Basal Secretion:

No significant difference was found between the breeds in their basal secretion of FSH during all ages considered.

GnRH Effects:

Angus (AN) (Fig 1) were notably higher in their response to GnRH at 3 and 5 mo ($p<0.0014$) with Crossbreds (XB) exceeding Polled Herefords (HP) ($p<0.02$). Polled Herefords were least responsive to GnRH at 3 & 5 mo, but were equal to the other breeds at 7 & 12 mo ($p>0.10$). Angus were significantly higher than XB at 7 mo ($p<0.0001$, Fig 2). No notable interbreed difference was detected in GnRH stimulated

release of FSH at 9 or 11-12 mo and since no significant change was found by 12 mo of age, the data from 11 and 12 mo were combined.

Age Effects:

Across breeds, basal secretion of FSH was numerically highest at 3 mo & declined slightly thereafter (Fig. 1 & 2). The response to GnRH increased in the HP at 7 mo ($p<0.05$), but

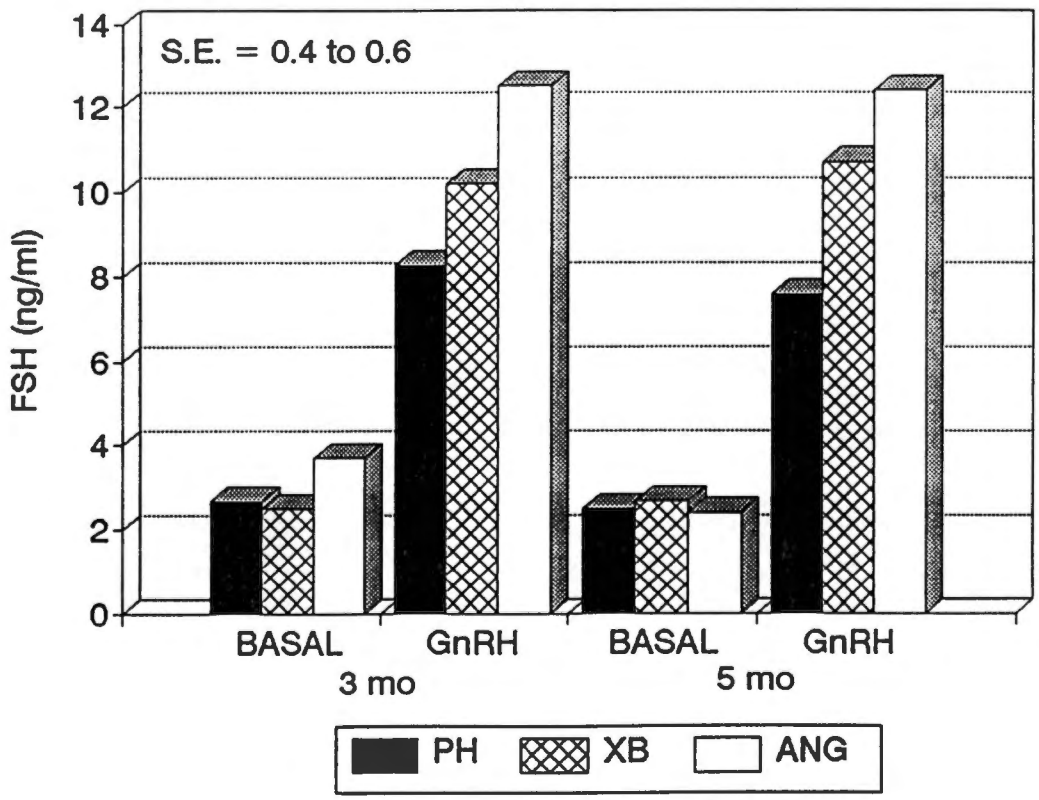

Fig 1 Follicle Stimulating Hormone secretion in the prepuberal heifer (3-5 mo)

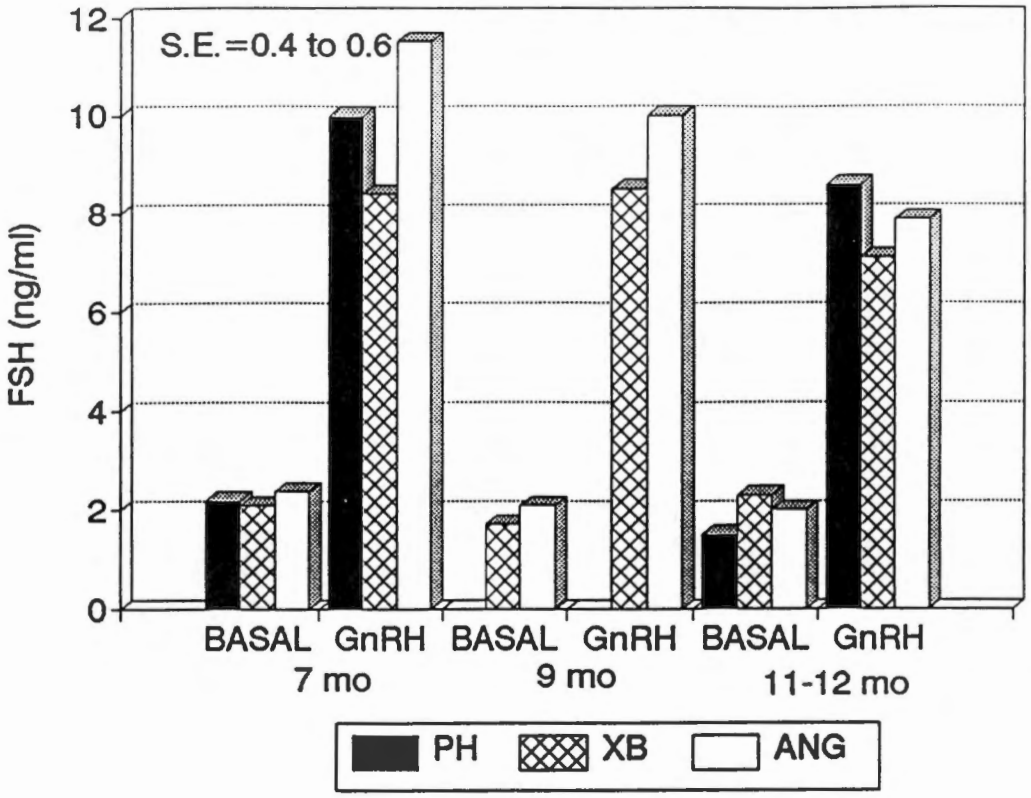

Fig 2 Follicle Stimulating Hormone secretion in the prepuberal heifer (7-12 mo)

a decrease occurred in the XB and AN ($p<0.05$). The GnRH response decreased between 7 and 12 mo in all breeds ($p<0.01$). Thus, the peak response to GnRH was observed from 3 to 5 mo for both XB and AN, while the maximum response by HP was observed at 7 mo.

LUTEINIZING HORMONE

Basal Secretion:

No significant difference was found in basal secretion of LH among the three breeds at any age. However, the HP were numerically higher than the other breeds at 5 and 12 mo of age (Fig. 3 & 4).

GnRH Effects:

At 3 mo of age (Fig. 3), AN had a greater response to GnRH than the other two breeds ($p<0.0001$). The HP were least

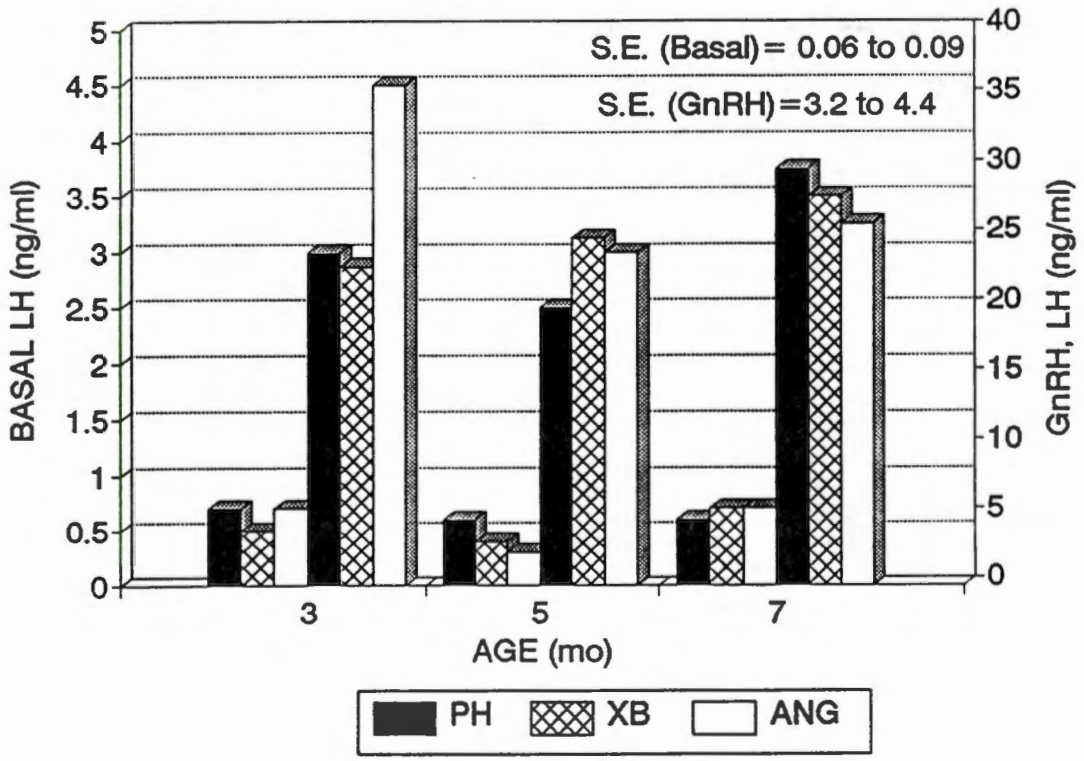

Fig 3 Luteinizing Hormone secretion in the puberal heifer. (3-7 mo)

responsive to GnRH at 5 mo ($p<0.03$) and no significant breed effects were found at the other ages.

Age Effects:

Basal secretion did not change appreciably with age, but an apparent increase was observed at 12 mo (Fig. 4). With the exception of the response of the AN at 3 mo (Fig. 3) the interbreed GnRH response was at a high at 9 mo, but not greatly different from that seen at 11 and 12 mo (Fig. 4).

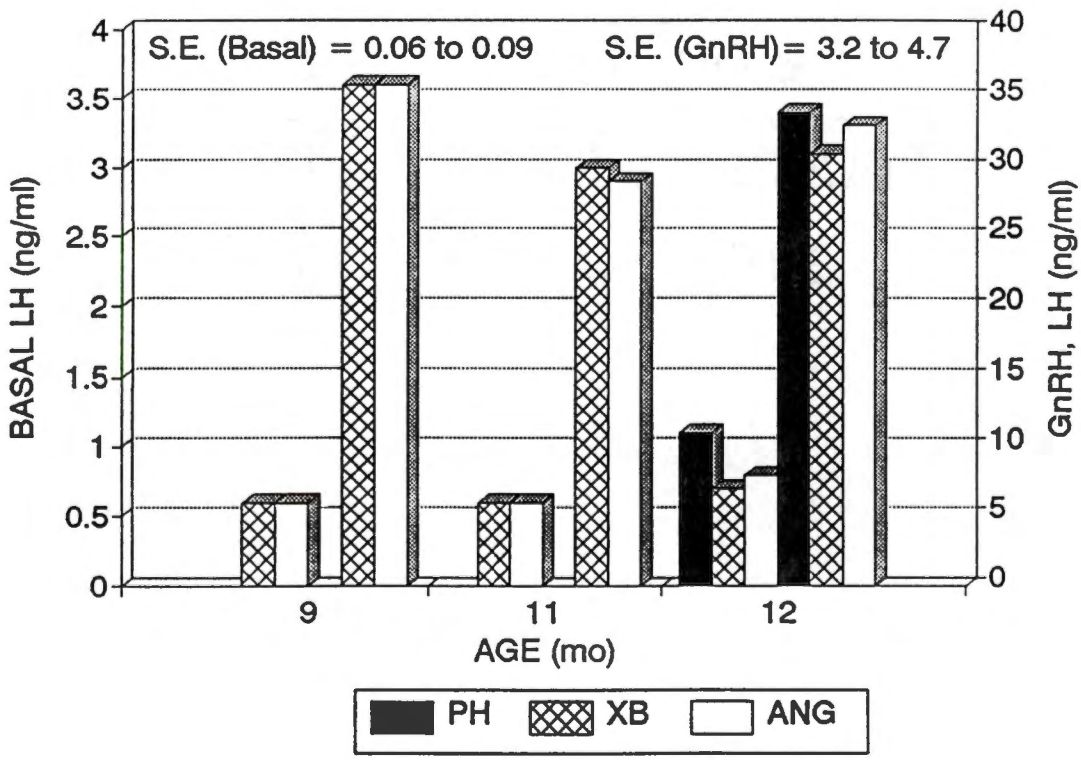

Fig 4 Luteinizing Hormone secretion in the puberal heifer. (9-12 mo)

PROGESTERONE

Basal Secretion:

At 3, 5 and 7 mo (Fig. 5) no significant difference was found between HP and XB. Angus, however, were superior in basal P_4 secretion at 3 mo of age ($p<0.05$). Angus were also distinctive at 5 mo, but at this age they were significantly lower than both HP ($p<0.001$) and XB ($p<0.01$) while being no different from the other two breeds in basal secretion at 7 mo.

Since P_4 values of 1,000 pg/ml or more signal the presence of a CL these values were eliminated from the analysis. This eventuality occurred between 9 and 12 mo. We were unable to obtain blood samples for HP at 9 and 11 mo of age. The other 2 breeds exhibited no significant difference in basal values at 9, 11 and 12 mo (Fig 6). At 9 mo, 43% of the XB had P_4 concentrations greater than 1,000 pg/ml while only 20% of the AN herd was exhibiting P_4 values over 1,000 pg/ml. At 11 mo, 10 of the 14 XB heifers (or 71%) had P_4 values over 1,000 pg/ml, while only 11 of the 25 ANG (or 44%) had ovulated. Basal secretion in HP at 12 mo (Fig. 6) was significantly greater than both XB and ANG ($p<0.05$). However, 57% of the XB group had P_4 values above 1,000 pg/ml with AN having 39% above 1,000 pg/ml, while only one HP heifer had P_4 values that high.

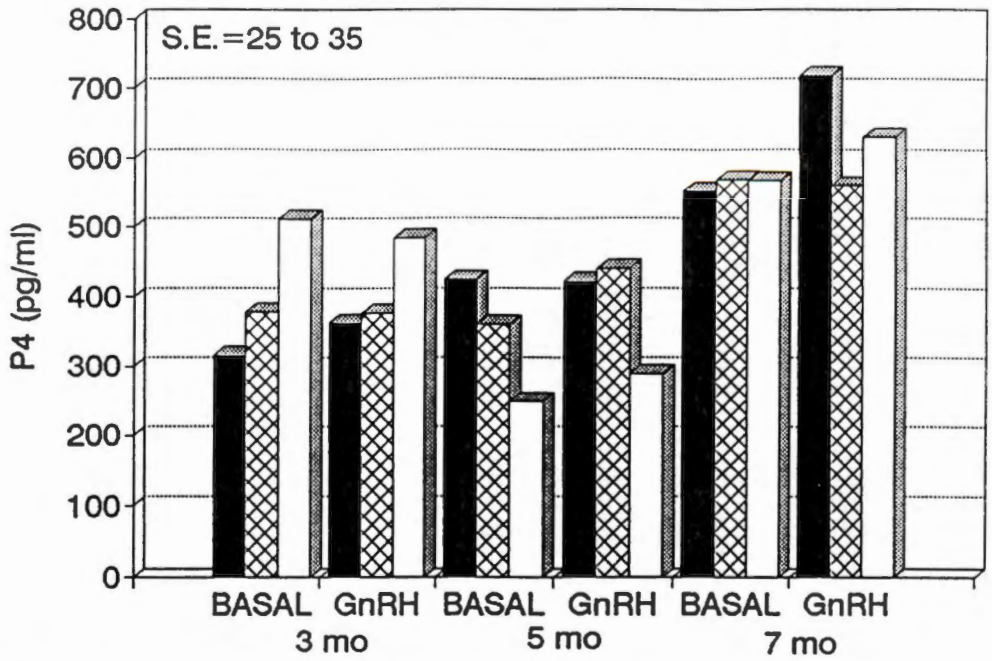

Fig. 5 Progesterone secretion in the prepuberal heifer (3-7 mo).

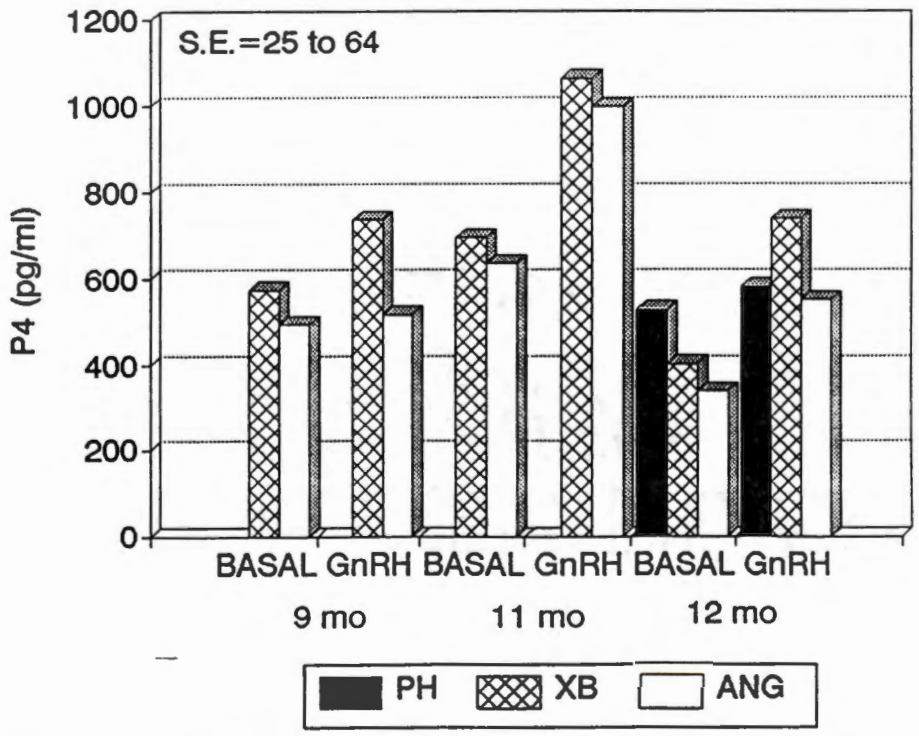

Fig. 6 Progesterone secretion in the prepuberal heifer. (9-12 mo)

GnRH Effects:

Detectable GnRH effects on P_4 secretion were modest to nonexistent at 3 through 7 mo, but thereafter (9-12 mo) the GnRH effect was sizeable and in most cases statistically significant.

Angus showed superiority in their response to GnRH at 3 mo (Fig. 5, $p<0.05$), while also being least in their response at 5 mo ($p<0.002$). Polled Herefords exhibited a noticeable increase over the other two breeds in response to GnRH at 7 mo (Fig. 5). Crossbreds (Fig. 6) were statistically highest at 9 and 12 mo ($p<0.01$) with no difference between the breeds at 11 mo.

Age Effects:

Both basal & GnRH values increased significantly at 7 mo and with one notable exception (the GnRH response at 11 mo) remained essentially unchanged through 12 mo (Fig. 5 & 6).

Thus 6 out of 14 XB heifers exhibited signs of puberty at 9 mo of age, 11 out of 25 AN at 11 mo and 1 out of 14 HP at 12 mo.

ANDROSTENEDIONE (Δ_4A)

Basal Secretion:

No significant difference in basal androstenedione secretion was observed between PH and XB at 3 and 5 mo of age. Angus, however, exceeded ($p<0.01$) the other two breeds at these same ages (Fig. 7). The ninth through the twelfth

months were dominated by the XB; the HP produced the least amount of Δ_4A at 12 mo.

GnRH Effects:

As with P_4, GnRH had no major effect on androstenedione secretion (Fig. 7 & 8). However, either numerically or statistically significant ($p<0.05$) increases were seen in the ANG at 3, 5, and 7 mo, but a GnRH effect was seen in HP & XB only at 7 and 12 mo. Crossbreds, however, secreted far more Δ_4A than the other two breeds between 9 and 12 mo ($p<0.05$) and HP, in general, secreted the least (Fig. 7 & 8).

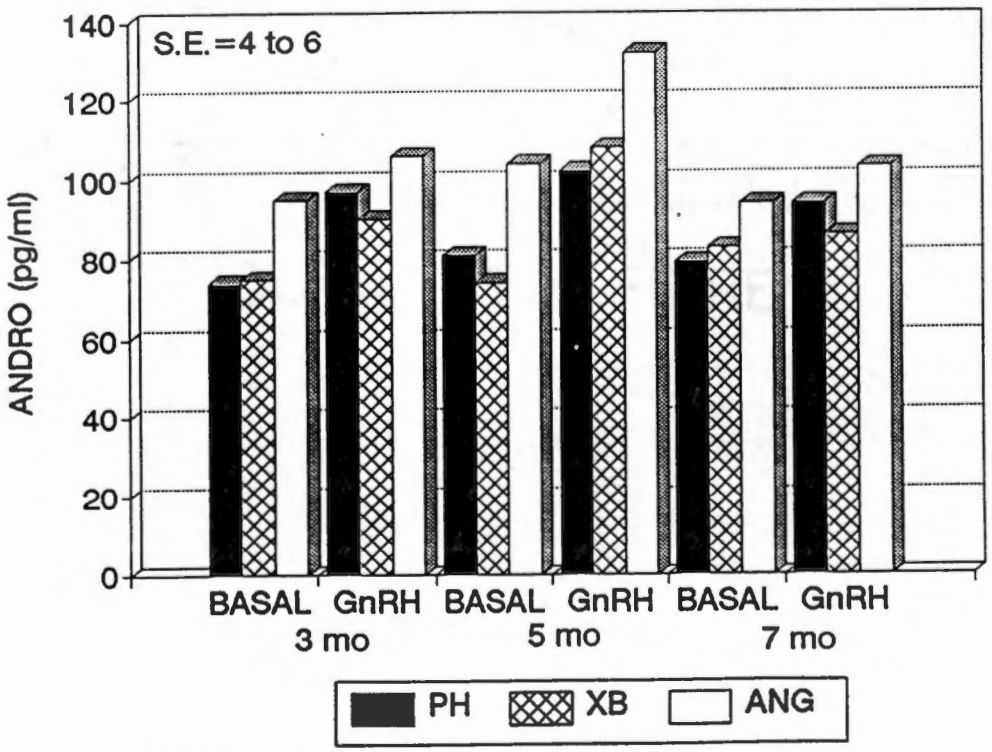

Fig. 7 Androstenedione secretion in the prepuberal heifer. (3-7 mo)

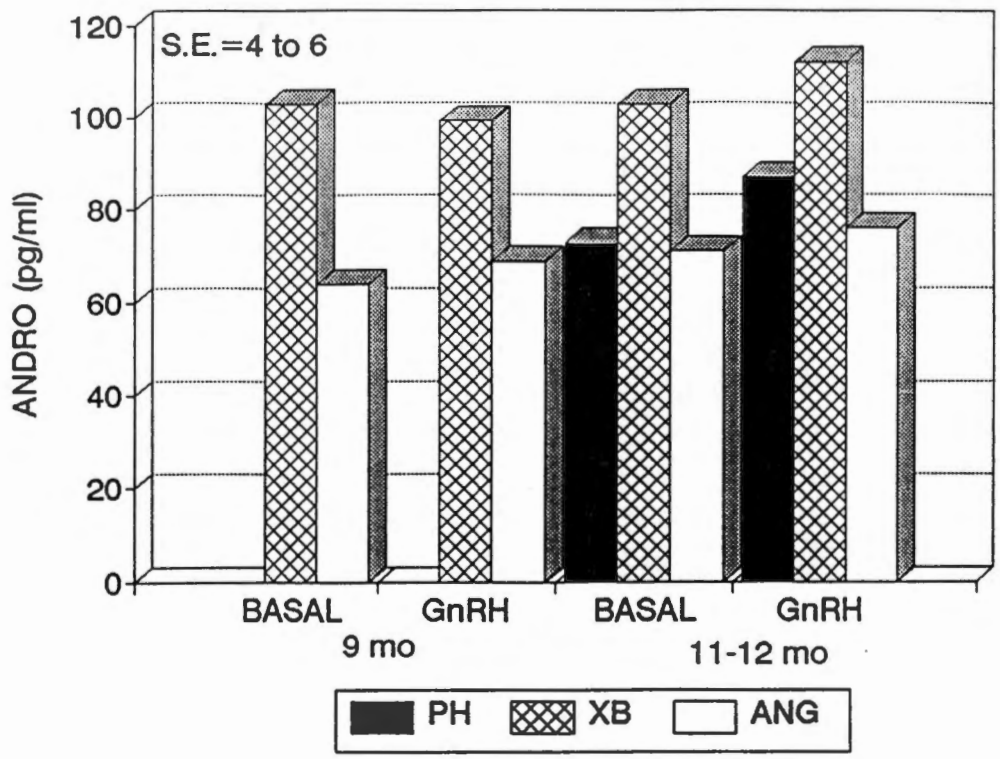

Fig. 8 Androstenedione secretion in the prepuberal heifer. (9-12 mo)

Age Effects:

No dramatic effect of age on Δ_4A secretion was exhibited. A peak apparently occurred at 5 mo (Fig. 7) followed by a decline in Δ_4A secretion at 7 mo with no significant change thereafter (Fig. 8).

TESTOSTERONE

Basal Secretion:

Crossbreds secreted far more T ($p<0.01$) than the other breeds at all ages (Fig. 9 & 10). Polled Herefords exceeded AN at 3 mo ($p<0.01$), but at other ages the differences between these breeds were not great.

GnRH Effects:

With the exception of the HP, GnRH, generally, had a major effect on T secretion at all ages (Figs. 9 & 10). The magnitude of the effect was greatest in the XB and least in the HP. A suggestion of a response to GnRH was seen at 12 mo, but otherwise the secretion of T by HP was not responsive to GnRH.

Age Effects:

Polled Herefords showed no significant change with age in either their basal or GnRH augmented secretion.

A steady increase in T secretion was, however, observed in XB heifers between 3 and 9 mo ($p<0.0001$). A downturn occurred at 11 mo ($p<0.0001$) with no significant change at 12 mo of age. The response to GnRH did not change between 3 and 5 mo and the response peaked at 9 mo ($p<0.0001$). Basal and GnRH secretion in the AN showed a pattern similar to the XB.

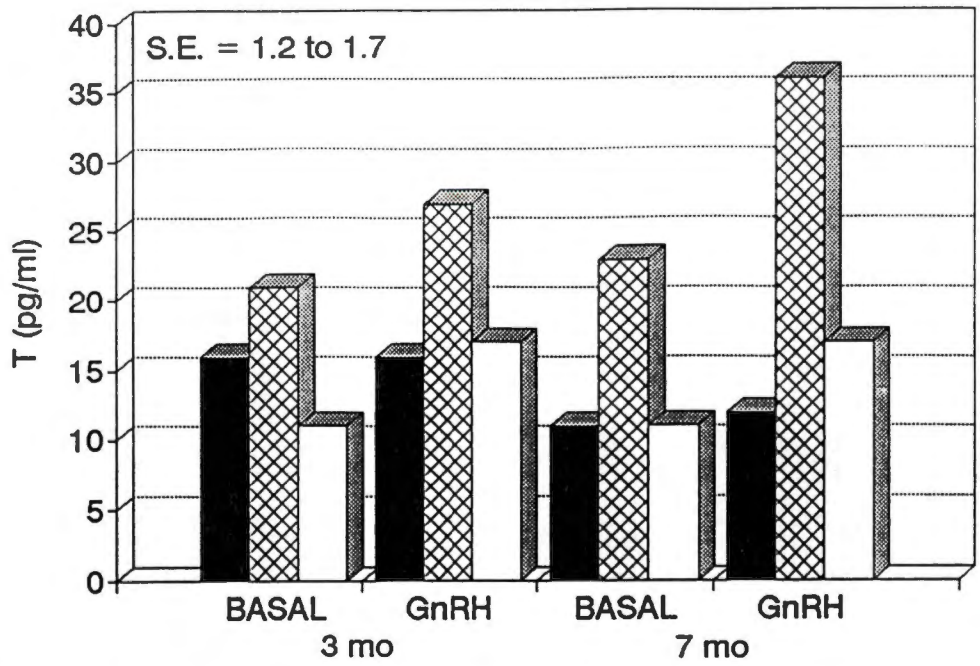

Fig. 9 Testosterone secretion in the prepuberal heifer. (3-7 mo)

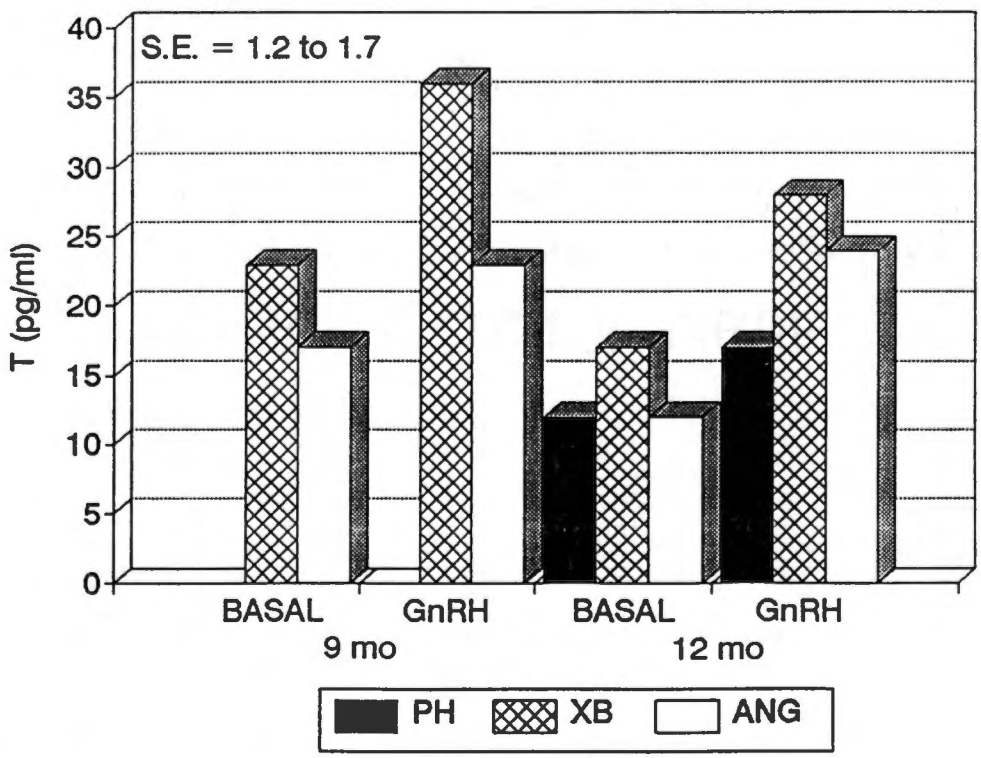

Fig. 10 Testosterone secretion in the prepuberal heifer. (9-12 mo)

ESTRADIOL

Basal Secretion:

No significant difference in basal estradiol secretion was detected among the three breeds of heifers at 3, 5, 7, 9 and 11 mo of age ($p<0.10$). Polled Herefords were numerically lower in basal secretion at 5 mo and statistically lower ($p<0.01$) at 12 mo. Angus were numerically superior at 5 and 7 mo, but as puberty approached, XB were highest at 9 and 11 mo but not different from AN at 12 mo ($p>0.10$).

GnRH Effects:

GnRH caused a major and statistically significant ($p<0.01$) increase in E_2 secretion at all ages and in all breeds (Fig. 11, 12 & 13). The response of the HP was less than the other breeds at 12 mo ($p<0.01$), but otherwise there were no significant differences. As was the case with basal secretion, the response of the XB at 9 and 11 mo was greater than that of the ANG.

Age Effects:

Neither basal nor GnRH affected secretion changed appreciably between 3 & 9 mo., but at 11 & 12 mo both basal & GnRH secretion increased ($p<0.01$, Figs. 11, 12 & 13).

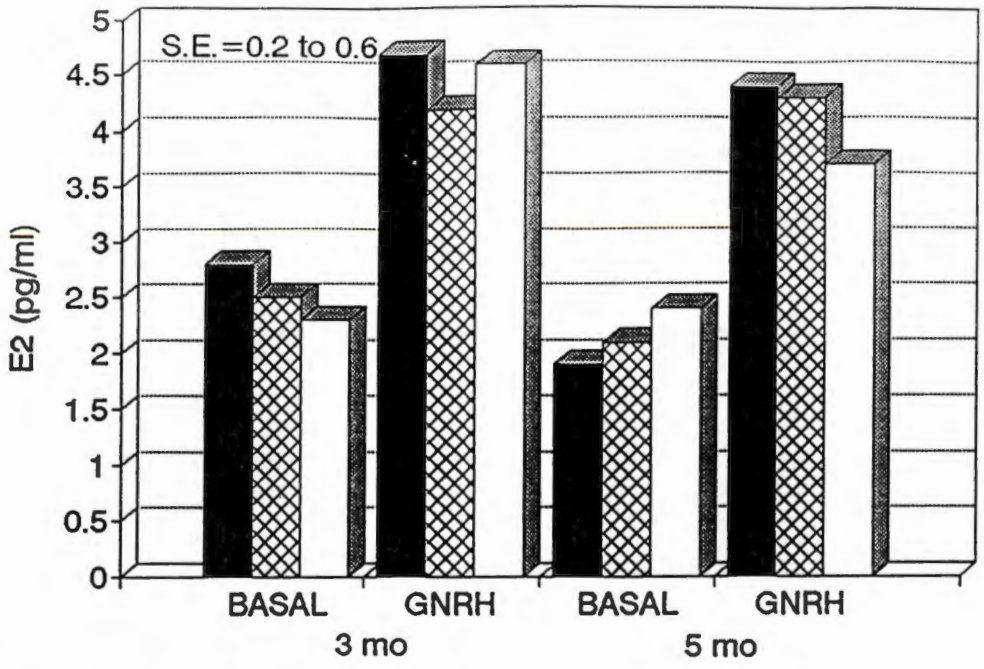

Fig. 11 Estradiol secretion in the prepuberal beef heifer (3-5 mo)

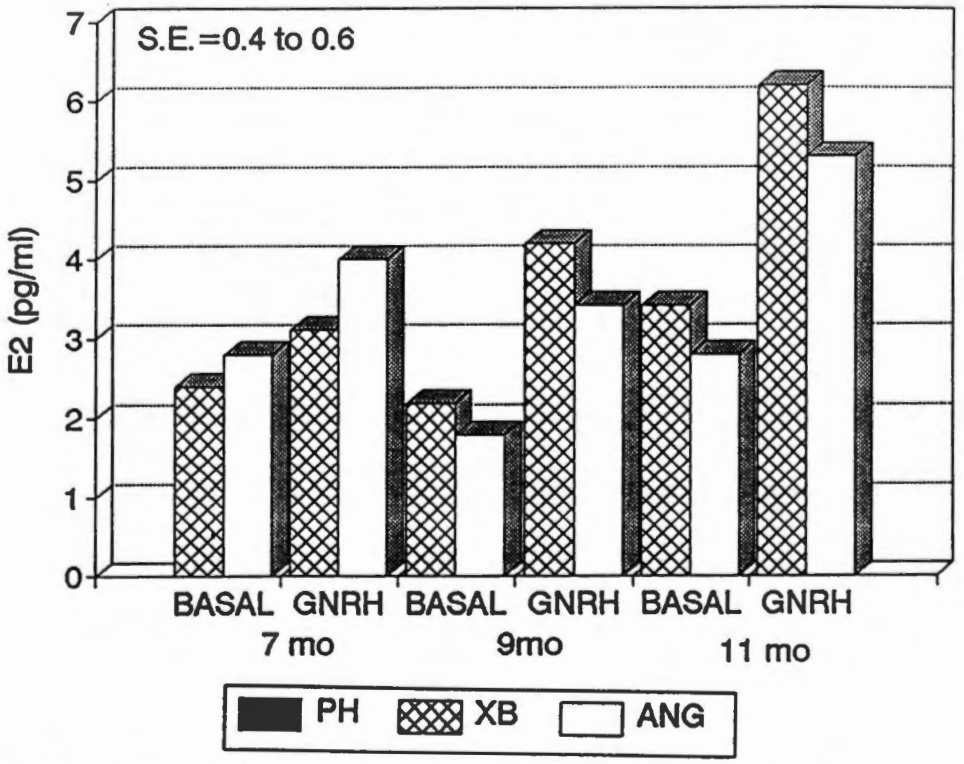

Fig. 12 Estradiol secretion in the prepuberal beef heifer (7-11 mo)

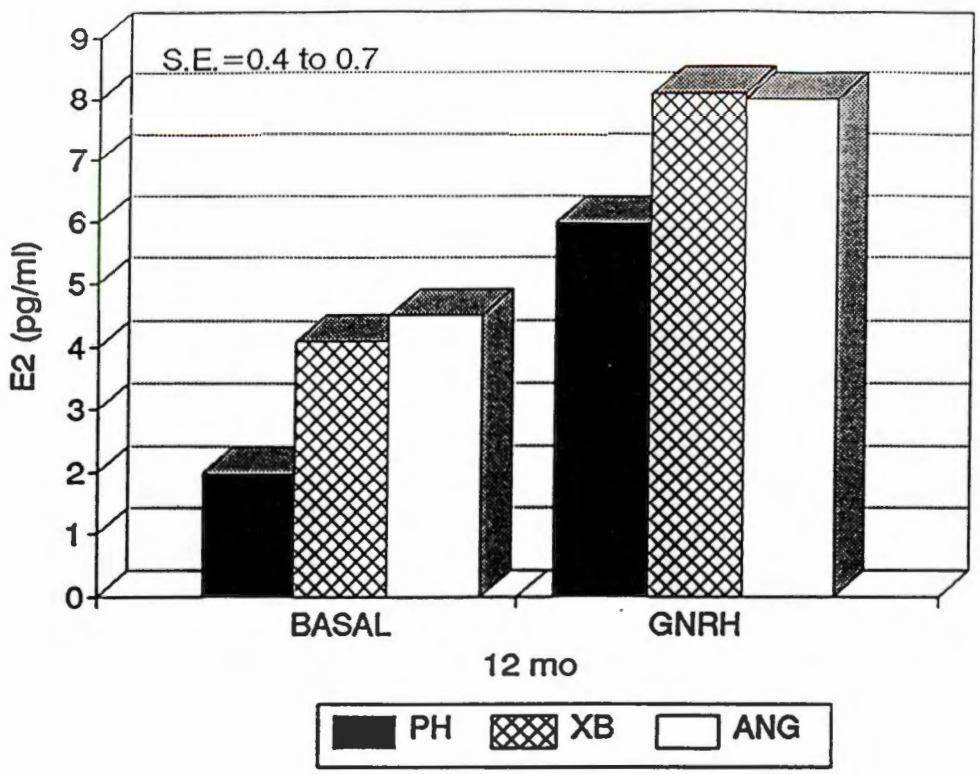

Fig. 13 Estradiol secretion in the prepuberal beef heifer (12 mo)

RELATIONSHIP BETWEEN HORMONES

In all breeds androstenedione increased from 3 to 5 mo then decreased at 7 mo of age. Progesterone, on the other hand, exhibited an inverse relationship to Δ_4A. Androstenedione, E_2 and LH showed similar patterns of change from 7-12 mo of age. Patterns of increases and decreases for E_2 and LH followed similar patterns at all ages considered.

from 7-12 mo of age. Patterns of increases and decreases for E_2 and LH followed similar patterns at all ages considered.

AGE AT OVULATION (EARLY vs LATE)

At 9 mo of age 43% of the XB and 8% of the AN exhibited P_4 values greater than 1 ng/ml and presumably had ovulated, while at 11 mo 71% and 32% of the respective breeds had values that high. Only one of the HP had apparently ovulated by 12 mo of age.

To determine if early ovulators were hormonally different from the late ovulators, heifers were assigned to two groups (group 1=early, group 2=late) and statistically contrasted for body weight and hormone secretion.

Weights were not significantly different between the two groups ($p>0.10$). The groups differed at 9 and 11 mo of age in both basal and GnRH stimulated secretion of Δ_4A ($p<0.01$), with group 1 being greater than group 2. Group 1 was also significantly higher in testosterone secretion at 7 mo of age ($p<0.01$). No significant difference in P_4 secretion between the two groups was found.

Group 1 had significantly higher basal and GnRH stimulated E_2 secretion at 3 mo of age ($p<0.01$). At all other ages E_2 tended to be higher in group 1, but, the differences were not significant ($p>0.10$).

DISCUSSION

The results show that Crossbred heifers significantly exceed Angus and Polled Herefords in birth, weaning and yearling weights ($p<0.003$) with no difference between the other two breeds. These results are consistent with that of Bailey (1988), Williams (1991) and Wiltbank and associates (1966). Weaning weights at 7 mo for XB heifers (250 kg) were within the range of weights observed in other XB heifers at the time of puberty (Wiltbank et al., 1966).

Yearling weights for XB (312 kg) far exceeded the other two breeds with only three kg separating the AN & HP (269 vs 266 kg). The lower yearling weights did not differ greatly from the weights of other HP (249-254 kg, Arije and Wiltbank, 1971) and ANG (251 kg, Wiltbank, 1966) reported at puberty .

Polled Herefords had basal LH values at 3, 5 and 12 mo numerically higher than the other two breeds. This, along with lower estrogen at 5 and 12 mo indicates retarded follicular development. Because of the low level of E_2 a corresponding increase in LH secretion and an increase in age at first ovulation was observed. Only one HP heifer had apparently ovulated by 12 mo of age. Therefore, the reduced reproductive capacity (unpublished) of this particular HP group is also associated with an increased age at first ovulation.

Crossbred heifers showed a greater secretion of

testosterone at all ages (Fig. 9 & 10). The source of this T appears to be ovarian in nature as, after stimulation with GnRH, T increased significantly at all ages. They were also significantly greater in their secretion of basal and GnRH stimulated Δ_4A from 9-12 mo of age. Crossbreds had numerically lower basal FSH at 3, 7 and 11 mo and lower basal LH at 3 and 12 mo. This suggests an enhanced ovarian development resulting in the higher estrogen values observed in XB at 9 & 11 mo. The reduced gonadotropin level and increased androgen and estrogen values indicate early ovarian development resulting in an earlier age at puberty in the XB. Forty-three percent of XB heifers had ovulated at 9 mo. In lambs, rats and humans, both testosterone and E_2 are capable of stimulating Growth Hormone (GH) release from the anterior pituitary. Testosterone also stimulates skeletal growth (Young et al., 1989; Simard et al., 1986; Shirasu et al., 1990). This may be the case in bovine as well, since those heifers with the highest T (at all ages) and Δ_4A (9-12 mo) (the XB) were consistently heavier than the other two breeds.

Angus were characterized by hormonal values that were superior to the HP but not the XB. The AN were numerically higher than the other two breeds in E_2 secretion at 5 and 7 mo and Δ_4A at 3 and 7 mo. As stated earlier, the higher E_2 & Δ_4A is indicative of early follicular growth resulting in an earlier age at ovulation (32% of the group had ovulated by 11 mo).

Luteinizing hormone, as well as FSH, release after GnRH was exceptional in the AN at 3, 5, 7 and 9 mo with XB exceeding the HP response at these same ages. This would indicate that the ability of the pituitary to respond to GnRH is also delayed in the HP.

There was only a modest response in the secretion of P_4 and Δ_4A after an injection of GnRH (Figs. 5, 6, 7 & 8). The release of E_2 & T from the ovaries, however, demonstrates that ovarian follicles are present & responsive in all breeds as early as 3 mo. The adrenal glands are, however, a major contributor to the concentrations of both P_4 and Δ_4A in peripheral blood (Butler et al., 1983; Jopson, et al., 1990). An ACTH injection resulted in a significant increase in P_4 and Δ_4A while having no effect on E_2 & T (Hillyer and Erickson, unpublished data). Thus, in excitable animals, the contribution from the adrenals could mask the GnRH effects on the P_4 & Δ_4A secretion of the ovary. Since E_2 & T are not secreted in measurable amounts by the adrenal, a clear GnRH response was seen at all ages. Patterns of change observed in this study revealed an apparent inverse relationship between Δ_4A and P_4 (Fig. 5, 6, 7 & 8) while Δ_4A, E_2 and LH were apparently reciprocally related (Figs. 7, 8, 11 & 12) near puberty. This is consistent with the results of other researchers who reported that Δ_4A is able to inhibit LH and P_4 in ewes (Philipon, 1989), gilts (McKinnie, 1988) and heifers (D'Occhio, 1988). Immunization against Δ_4A in sheep has

resulted in either a decrease (Philipon, 1989) or no change in the secretion of FSH (Martensz and Scaramuzzi, 1979; Pathiraja et al., 1984). Testosterone had no effect on FSH secretion (Philipon, 1989) while exhibiting an inhibitory effect on LH in heifers (Price, 1987).

Values for all hormones studied were within the ranges previously reported by other researchers. Peterson and associates (1978) reported Δ_4A in dairy cows, at estrus or the at the time of luteolysis, varied between 5-60 pg/ml, with testosterone ranging from 5-80 pg/ml. Kanchev and Dobson (1976) reported Δ_4A during the bovine estrus cycle to range from 80-100 pg/ml which was consistent with Shemesh and Hansel's findings (1974). In 1976, Saba and associates reported 58-60 pg/ml to be the range for Δ_4A in one year old heifers. Results for Δ_4A over the years remain fairly consistent, however, the differences observed in the ranges by the various researchers could be attributed to the fact that Δ_4A fluctuates considerably from day to day in the bovine (Kanchev, 1976).

Kotwica & Williams (1982) reported E_2 values of 6.4-8.3 pg/ml during the bovine estrus cycle, but, Kesler et al. (1977) reported the concentrations of E_2 to be much lower (1.0-3.6 pg/ml).

Progesterone in the prepuberal heifer averaged 300 pg/ml (Gonzalez-Padilla et al., 1975) and LH ranged from .48 to .88 ng/ml (Mathison, 1986). Values of FSH found in this study

were lower than those observed by other researchers. Turzillo and Fortune (1990) and Adams and associates (1992) reported FSH values to be 10-18 ng/ml and 9-12 ng/ml respectively. Values observed by Evans & co-workers (1992) (0.5 to 1.0 ng/ml), however, were lower than those reported here.

Early ovulators (P_4 > 1ng/ml) had significantly higher E_2 at 3 mo, higher Δ_4A at 9 & 11 mo and numerically higher E_2 at all other ages. This, in addition to the differences observed among breeds, indicate that concentrations of E_2 & androgens may reflect a propensity for an early age at puberty.

Conclusions:

Significant differences in prepuberal hormonal secretion exists among the 3 breeds tested. Polled Herefords secreted more gonadotropin and less E_2 than the other breeds and were older at puberty. Angus had higher Δ_4A early in life and lower gonadotropin levels than HP, but release of LH & FSH in response to GnRH was markedly higher in the AN. Crossbreds were significantly heavier at the three weighing periods, had significantly higher androgen levels and ovulated two mo earlier than AN. Consequently, androgens probably play an important role in the growth and early reproductive development of the beef heifer. In summation, prepuberal secretion of gonadotropins and gonadal steroids could be used as markers for reproductive potential.

LIST OF REFERENCES

Adams, G. P., R. L. Matteri, J. P. Kastelic, J. C. H. Ko and O. J. Ginther. 1992. Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. J. Reprod. Fertil. 94:177.

Ainsworth, L., B. K. Tsang, B. R. Downey and G. J. Marcus. 1990. The synthesis and actions of steroids and prostaglandins during follicular maturation in the pig. J. Reprod. Fert. Suppl. 40:137.

Amann, R. P. and O. A. Walker. 1983. Changes in the pituitary-gonadal axis associated with puberty in holstein bulls. J. Anim. Sci. 57:433.

Arije, G. F. and J. N. Wiltbank. 1971. Age and weight at puberty in hereford heifers. J. Anim. Sci. 33:401.

Babalola, G. O. and B. H. Shapiro. 1988. Correlation of follicular sterid hormone profiles with ovarian cyclicity in sows. J. Reprod. Fert. 84:79.

Bailey, C. M., D. R. Hanks, W. D. Foote and Y. O. Koh. 1988. Maternal characteristics of young dams representing bos X Taurus and bos indicus X bos Taurus breed types. J. Anim. Sci. 66:1144.

Berardinelli, J. G., R. A. Dailey, R. L. Butcher and E. K. Inskeep. 1979. Source of progesterone prior to puberty in beef heifers. J. Anim. Sci. 49:1276.

Blakely, J. and D. H. Bade. 1990. The Science of Animal Husbandry. 5th edition, Chapt. 3. Prentice-Hall, Inc., N.J.

Blouin, D. C. and A. M. Saxton. 1990. General Linear Mixed Models GLMM User's Manual. Louisiana State University, Baton Rouge.

Bolt, D. J. and R. Rollins. 1983. Development and application of a radioimmunoassay for bovine follicle-stimulating hormone. J. Anim. Sci. 56:146.

Britt, J. H. 1990. RIA for estradiol-17 beta. Reproductive Endocrinology Lab. Dept. of Animal Science, North Carolina State University.

Brown, N. S., S. R. Abbott and J. E. T. Corrie. 1983. some observations on the preparations, purification and storage of radioiodinated protein hormones. In: Immunoassays for clinical chemistry. W. M. Hunter and J. E. T. Corrie, Editors 2nd Edition, pp.167-276. Churchill Livingston, New York.

Bushmich, S. L., R. D. Randel, M. M. McCarter and L. H. Carroll. 1980. Effect of dietary monensin on ovarian response following gonadotrophin treatment in prepuberal heifers. J. Anim. Sci. 51:692.

Butler, W. R. , L. S. Katz, J. Arriola, R. A. Milvae and R. H. Foote. 1983. On the negative feedback regulation of gonadotropins in castrate and intact cattle with comparison of two FSH radioimmunoassays. J. Anim. Sci. 56:919.

Cato, R. L. 1986. Relationship between prepuberal gonadotrophin secretion and reproductive performance in the beef heifer. Masters Thesis, The University of Tennessee.

Cundiff, L. V. 1970. Experimental results on crossbreeding cattle for beef production. J. Anim. Sci. 30:694.

Day, M. L., K. Imakawa, P. L. Wolfe, R. J. Kittok and J. E. Kinder. 1987. Endocrine mechanisms of puberty in heifers. Role of hypothalmo-pituitary estradiol receptors in the negative feedback of estradiol on luteinizing hormone secretion. Biol. Reprod. 37:1054.

D'Occhio, M. J., D. R. Gifford, R. M. Hoskinson, T. Weatherly and B. P. Setchell. 1988. Gonadotropin secretion and ovarian responses in prepubertal heifers actively immunized against androstenedione and oestradiol-17β. J. Reprod. Fert. 83:159.

Erickson, B. H. 1966. Development and senescence of the postnatal bovine ovary. J. Anim. Sci. 25:800.

Evans, A. C. O., G. P. Adams and N. C. Rawling. 1992. Follicular waves and gonadotropins in 36 wk old heifers. 25th Annual SSR meeting.

Gonzalez-Padilla, E., J. N. Wiltbank and G. D. Niswender. 1975. Puberty in beef heifers. 1. The interrelationship between pituitary, hypothalamic and ovarian hormones. J. Anim. Sci. 40:1091.

Howe, G. R., D. L. Black, R. C. Filey and W. G. Black. 1962. Ovarian activity in prepuberal dairy cows. J. Anim. Sci. 21:82.

Imakawa, K., M. L. Day, M. Garcia-Winder, D. D. Zalesky, R. J. Kittok, B. D. Schanbacher and J. E. Kinder. 1986. Endocrine changes during restoration of estrous cycles following induction of anestrus by restricted nutrient intake in beef heifers. J. Anim. Sci. 63:565.

Jackson, J. A., G. M. Butterstein and E. D. Albrecht. 1989. The effect of estrogen on androstenedione production by rat placental cells in vitro. Biol. Reprod. 40:74.

Jopson, N. B., M. W. Fisher and J. M. Suttie. 1990. Plasma progesterone concentrations in cycling and in ovariectomised red deer hinds: the effect of progesterone supplementation and adrenal stimulation. Anim. Reprod. Sci. 23:61.

Kanchev, L. N. and H. Dobson. 1976. Plasma concentration of androstenedione during the bovine oestrous cycle. J. Endocrin. 71:351.

Kesler, D. J., H. A. Garverick, R. S. Youngquist, R. G. Elmore and C. J. Bierschwal. 1977 Effect of days postpartum and endogenous reporductive hormones on GnRH [gonadotropin-releasing hormone]-induced LH [luteinizing hormone]-release in dairy cows. J. Anim. Sci. 45:797.

Kinder, J. K., M. L. Day and R. J. Kittok. 1987. Endocrine regulation of puberty in cows and ewes. J. Reprod. Fert. Suppl. 34:167.

Kotwica, J and G. L. Williams. 1982. Relationship of plasma testosterone concentrations to pituitary-ovarian hormone secretion during the bovine estrous cycle and the effects of testosterone propionate administered during luteal regression. Biol. Reprod. 27:790.

Martensz, N. D. and R. J. Scaramuzzi. 1979. The plasma concentrations of luteinizing hormone, follicle-stimulating hormone and progesterone during the breeding season in ewes immunized against androstenedione and testosterone. J. Endocrin. 81:249.

Mathison, B. D., H. M. Schoenemann and J. J. Reeves. 1986. Differences in immunological and biological activity of serum luteinizing hormone during the bovine estrous cycle. Domestic Anim. Endocrin., College of Veterinary Medicine, Auburn University. Jan., v. 3.

McCarter, M. N. , D. S. Buchanan and R. R. Frahm. 1991. Comparison of crossbred cows containing various proportions of brahman in spring- or fall-calving systems: IV. Effects of genotype X environment interaction of lifetime productivity of young cows. J. Anim. Sci. 69:3977.

McKinnie, M. R., J. H. Britt and K. L. Esbenshade. 1988. Ovarian function and hormone secretion of gilts actively immunized against androstenedione. J. Anim. Sci. 66:3131.

Meinert, R. A., C.-M. J. Yang, A. J. Heinrichs and G. A. Varga. 1992. Effect of Monensin on growth, reproductive performance, and estimated body composition in holstein heifers. J. Dairy Sci. 75:257.

Moran, C., J. F. Quirke and J. F. Roche. 1989. Puberty in heifers: A review. Anim. Reprod. Sci. 18:167.

Neumann, A. L. 1977. Beef Cattle. 7th edition. John Wiley & Sons, Inc., N. Y., N.Y.

Niswender, G. D., L. E. Jr. Richert, A. R. Jr. Midgley and A. V. Nalbandov. 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology 84:1166.

Pathiraja, N., W. R. Carr, M. Fordyce, J. Forster, R. B. Land and B. A. Morris.. 1984. Concentration of gonadotrophins in the plasma of sheep given gonadal antisera to raise ovulation rate. J. Reprod. Fertil. 72:93.

Peacock, F. M., M. Kroger, W. G. Kirk, E. M. Hodges and A. C. Warnick. 1971. Reproduction in brahman, shorthorn and crossbred cows on different pasture programs. J. Anim. Sci. 33:458.

Peterson, A. J., R. J. Fairclough and J. F. Smith. 1978. Radioimmunoassay of androstenedione and testosterone in cow plasma at the time of luteolysis and oestrus. J. Reprod. Fert. 52:127.

Philipon, P., A. L. Prieur and M. A. Driancourt. 1989. Alterations in Gonadotrophin secretion and ovarian sensitivity to gonadotrophins induced by active immunization against androstenedione. Anim. Reprod. Sci. 19:53.

Price, C. A., B. A. Morris and R. Webb. 1987. Reproductive and endocrine effects of active immunization against a testosterone conjugate in the heifer. J. Reprod. Fert. 81:149.

Putnam, C. D., D. W. Brann and V. B. Mahesh. 1991. Acute activation of the adrenocorticotropin-adrenal axis: effect on gonadotropin and prolactin secretion in the female rat. Endocrinology 128:2558.

Reynolds, W. L., R. A. Bellows, J. J. Urick and B. W. Knapp. 1986. Crossing beef X beef and beef X brown swiss: Pregnancy rate, calf survival, weaning age and rate. J. Anim. Sci. 63:8.

Saba, N., N. R. Cunningham and P. G. Miller. 1975. Plasma progesterone and testosterone concentrations in freemartin heifers. J. Reprod. Fertil. 45:37.

Sacco, R. E., J. F. Baker, T. C. Cartwright, C. R. Long and J. O. Sanders. 1989. Lifetime productivity of straightbred and F1 cows of a five-breed diallel. J. Anim. Sci. 67:1964.

Schillo, K. K., P. J. Hansen, L. A. Kamwanja, D. J. Dierschke and E. R. Hansen. 1983. Influence of season on sexual development in heifers: Age at puberty as related to growth and serum concentrations of gonadotropins, prolactin, thyroxine and progesterone. Biol. Reprod. 28:329.

Shemesh, M. and W. Hansel. 1974. Measurement of bovine plasma testosterone by radioimmunoassay (RIA) and by a rapid competitive protein binding (CPB) assay. J. Anim. Sci. 39:720.

Shirasu, K. W., E. Stumpf and M. Sar. 1990. Evidence for direct action of estradiol on growth hormone-releasing factor (GRF) in rat hypothalamus: Localization of [^{3}H]estradiol in GRF neurons. Endocrinology 127:344.

Simard, J., J. Hubert, T. Hosseinzadeh and F. Labrie. 1986. Stimulation of growth hormone release and synthesis by estrogens in rat anterior pituitary cells in culture. Endocrinology 119:2004.

Staigmiller, R. B., R. E. Short and R. A. Bellows. 1979. Induction of LH surges with 17β-estradiol in beef heifers: An age dependent process. Therio. 11:453.

Turzillo, A. M. and J. E. Fortune. 1990. Suppression of the secondary FSH surge with bovine follicular fluid is associated with delayed ovarian follicular development in heifers. J. Reprod. Fertil. 89:643.

Van Vleck, L. D., E. J. Pollack, E. A. B. Oltenacu. 1987. Genetics for the Animal Sciences. W. H. Freeman & Co.

Williams, A. R., D. E. Franke and A. M. Satxon. 1991. Genetic effects for reproductive traits in beef cattle and predicted performance. J. Anim. Sci. 69:531.

Wiltbank, J. N., K. E. Gregory, L. A. Swiger, J. E. Ingalls, J. A. Rothlisberger and R. M. Koch. 1966. Effects of heterosis on age & weight at puberty in beef heifers. J. Anim. Sci. 25:744.

Young, L. R. , S. Mesiano, R. Hintz, D. J. Caddy, M. M. Ralph, C. A. Browne and G. D Thorburn. 1989. Growth hormone and testosterone can independently stimulate the growth of hypophysectomized prepubertal lambs without any alteration in circulating concentrations of insulin-like growth factor. J. Endocrin. 121:563.

STUDY II

CORRELATION OF Δ_4A AND OTHER STEROID HORMONES WITH NUMBER OF OVARIAN FOLLICLES

ABSTRACT

A two year study was conducted, utilizing ovaries from 20 Angus (AN) in Year-1 and 10 AN and 8 Crossbred heifers (XB) (HPxAN) in Year-2. Ovaries were sectioned and their content of follicles (primary (PRIM), secondary (SEC) vesicular (VES)) and corpora lutea (CL) (active and regressed CL (RCL)) was quantified. Heifers were bled when their average age was 3, 5, 7, 9, 11 & 12 mo old. Assays (RIA) were conducted for the steroids progesterone (P_4), androstenedione (Δ_4A), estradiol (E_2) and testosterone (T). Testosterone was not assayed in Year-1. Correlations were conducted between basal and GnRH stimulated steroid concentrations in serum and number of ovarian follicles. Heifers with the greatest number of primary follicles had significantly higher Δ_4A at 9 and 11 mo. Year 2 heifers with the highest number of primary and secondary follicles also had the highest basal T at 7 mo ($p<0.0001$). A high correlation was observed between primary, secondary, growing, atretic and total vesicular follicle numbers ($r=.5$, $p<0.01$). Primary numbers were also associated with both basal and GnRH stimulated Δ_4A concentrations ($r=.4$, $p<0.01$).

These results indicate that high basal and GnRH stimulated T at 7 mo and Δ_4A at 9 and 11 mo could be used to identify high primary follicle numbers and the likelihood of a prolonged reproductive life in the beef heifer.

INTRODUCTION

Source of Δ_4A:

Androstenedione (Δ_4A) is a 19 carbon steroid hormone produced in the female by follicular theca cells (Erickson et al., 1985) and the zona reticularis of the adrenal cortex (Guyton, 1976; Griffin and Ojeda, 1988; Coffey, 1988; Takeda et al., 1990). Small vesicular and atretic follicles are the major ovarian contributors to Δ_4A secretion, with the large dominant follicle being primarily a secretor of estrogen (Campbell et al., 1990a; Ireland and Roche, 1983; Kruip and Dielman, 1985; McNatty et al., 1981; Spicer and Zinn, 1987). In cows, ewes and humans, a characteristic feature of atretic follicles is a high concentration of androgens in their follicular fluid (Prevost et al., 1989), which may be due to the fact that the theca layer is the last area affected by atresia (Wise, 1987). Hence, both normal and atretic follicles are capable of producing androstenedione which may provide a good index of ovarian follicular numbers.

Effects and Mode of Action:

Androstenedione elicits it's response by binding to it's specific receptor, which, like other steroid receptors, is located in the nucleus of the cell (Handa, 1987; Takeda et al., 1990).

Androgen receptors have been found to be widespread

throughout the male and female reproductive organs, liver, adrenal, kidney, muscle, pituitary and the brain (Coffey, 1988; Takeda et al., 1990). Ovarian androgen receptors in the primate are localized in both the theca and granulosa layers of growing and atretic follicles. Functional CL in the primate have both androgen and P_4 receptors, however, at the onset of luteolysis the number of P_4 receptors decline while the number of androgen receptors remain constant (Hild-Petito et al., 1991). This suggests androgens may play a significant part in luteal regression. The paracrine role androgens play in the ovary are not yet fully understood, but through the work of Henderson & Franchimont (1981) it was concluded that one specific role of ovarian androgens is to stimulate the production of inhibin from the granulosal cells in the bovine ovary. Pituitary androgen receptor numbers fluctuate during the estrous cycle; the largest number coinciding with elevations in serum estrogen concentrations (Handa, 1987). Estrogen may up-regulate androgen receptors within the pituitary and a potential exists for both estrogen and Δ_4A to act in synchrony in controlling pituitary gonadotropin secretion (Handa, 1987). A significant portion of Δ_4A is converted to estradiol through an aromatase enzyme complex activated by follicle stimulating hormone (FSH), with aromatization taking place in one of three areas: 1) granulosa cells, as a consequence of Δ_4A diffusion from the theca (Griffin and Ojeda, 1988), 2) the brain, principally

the diencephalon (Connolly et al., 1990; Roselli, 1987) and 3) adipose tissue (Roselli, 1987; Griffin and Ojeda; 1988).

Relationship With Other Steroids:

Secretion of bovine ovarian Δ_4A is enhanced through adenylate cyclase activated by luteinizing hormone (LH) (Griffin and Ojeda, 1988; McNatty et al., 1984; Erickson et al., 1985) and is positively correlated with estrogen at all stages of the estrous cycle (Campbell et al., 1990a; Campbell et al., 1990b; Fortune and Hansel, 1985). Both Δ_4A and E_2 are secreted episodically from ovarian follicles (Campbell et al., 1990a; Campbell et al., 1990b), however, the episodes are generally masked in the peripheral circulation because of a long half life of the steroid and insufficient magnitude of the pulse.

Androgen (both Δ_4A and testosterone) concentrations in the bovine increases 1-4 days prior to luteal regression. Spikes of Δ_4A & T also occur concurrently with the decrease in progesterone secretion on the day of estrus and one day prior to estrus respectively (Wise et al., 1982; Peterson et al., 1978; Kanchev et al., 1976). This characteristic rise and fall in androgens may be an indicator of waves of follicular growth and development (Wise et al., 1982).

Androstenedione has been reported to inhibit progesterone production by the CL in the ovary of primates (Hild-Petito et al., 1991) and pigs (Ainsworth et al., 1990).

A possible mechanism could be the inhibition of 3β-hydroxysteroid dehydrogenase activity (Hild-Petito et al., 1991) which converts pregnenolone to progesterone (Norman & Litwack, 1987). D'Occhio and associates (1988) reported that 5-6 mo old heifers had increased P_4 values when immunized against androstenedione, which is consistent with results in the gilt (McKinnie et al., 1988). Heifers immunized against Δ_4A also had increased LH secretion (Philipon et al., 1989; D'Occhio et al., 1988) suggesting that Δ_4A has an inhibitory effect on the hypothalamus and pituitary. Thus, immunization against Δ_4A would result in an increase in LH secretion and ultimately an increase in P_4 secretion. It has also been shown that immunization against androstenedione resulted in a significantly higher ovulation rate in heifers (Wise and Schanbacher, 1983), gilts (McKinnie et al., 1988) and ewes (Cameron et al., 1988).

Follicular Development:

In the bovine, the number of primordial follicles varies dramatically between animals of the same age; ovaries in 3 mo old calves contained 75,000 to 297,000 primary oocytes (Marden, 1953). Erickson (1966) reported the number of primordial follicles in heifers remains nearly constant from birth to four years of age, then proceeds to decline. Secondary and antral follicles are present at birth, but antral follicles do not exhibit a decrease in numbers until

approximately ten years of age (Erickson, 1966).

Objective:

The objective of this study was to quantify GnRH stimulated and nonstimulated hormonal secretion at various ages in the heifer and determine the extent to which these values were correlated with number of follicles in the postpuberal ovary.

MATERIALS AND METHODS

Experimental Design

A two year study was conducted during which blood serum was collected from 20 AN heifers during Year-1 and from 10 ANG and 8 XB heifers during Year-2. Heifers were bled when their average age was 3, 5, 7, 9, 11 and 12 mo. Utilizing protocols from study I, RIA's were conducted for P_4, Δ_4A and E_2. Testosterone was not assayed in Year-1.

Histological Techniques:

A total of 38 heifers (20 ANG from year 1, 10 ANG and 8 XB from year 2) were bilaterally ovariectomized via an incision in the left paralumbar fossa under local and spinal anesthesia. Ovaries were fixed in Bouin's Fixative, sectioned and stained with Hematoxylin and Eosin (H&E). Microscopically aided counts were made of primary, secondary, vesicular and atretic follicles. Number of corpora lutea (CL) and regressed CL (RCL) were also enumerated. Follicular numbers were correlated with hormonal data by utilizing Pearson's product-moment correlation coefficient adapted to Statistical Analysis System (SAS, 1985; SAS, 1989).

Statistical Analysis:

Correlations between ovarian morphology and hormones were computed on GLM provided by SAS (1985, 1989).

HORMONE SECRETION

Basal androstenedione (Fig 1) showed no appreciable change in secretion with age. Response to GnRH was greatest at 3 & 5 mo of age and the degree of the response declined with age thereafter.

Progesterone secretion (Fig 2) increased markedly between 5 & 7 mo and did not change significantly at 9 mo. Only a modest progesterone response to GnRH was noted and the pattern of the response closely followed basal secretion.

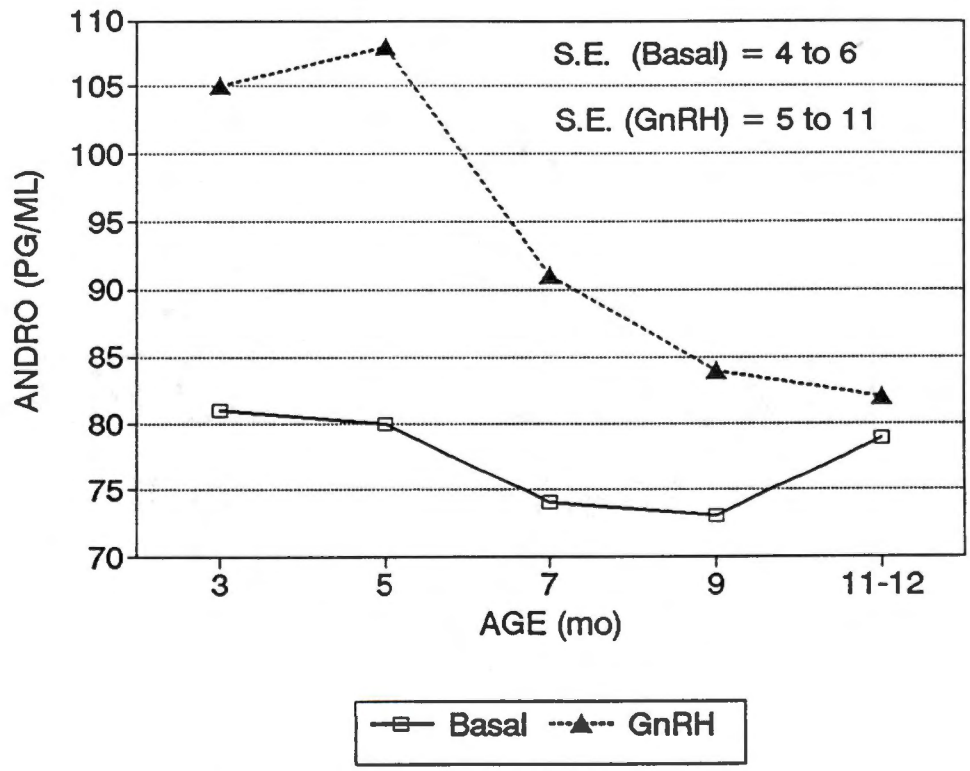

Fig 1. Androstenedione secretion in prepuberal heifers

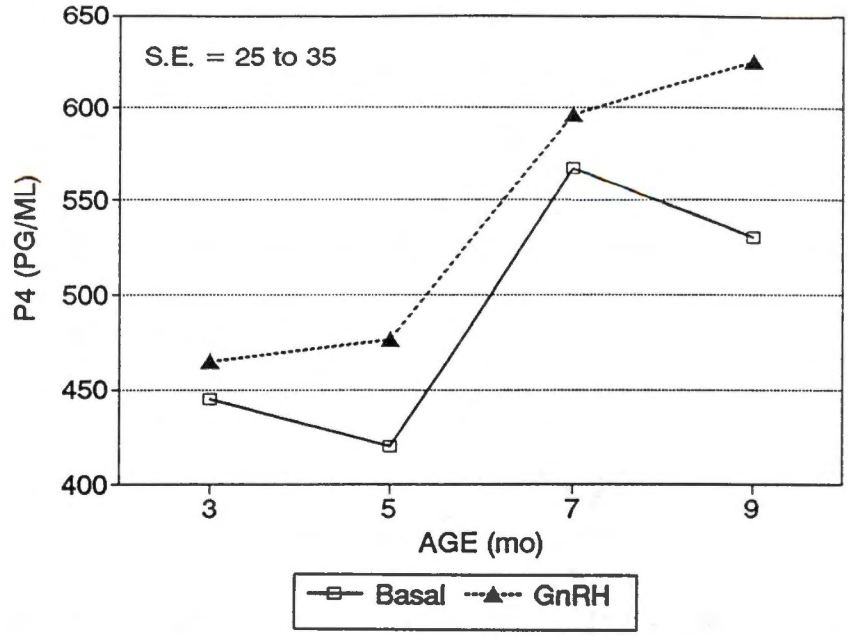

Fig 2. Progesterone secretion in prepuberal heifers

Basal testosterone secretion (Fig 3) increased with age through 9 mo and declined at 12 mo, but the inter-age differences were not significant. The amount of T released in response to GnRH was significant at all ages ($p<0.01$).

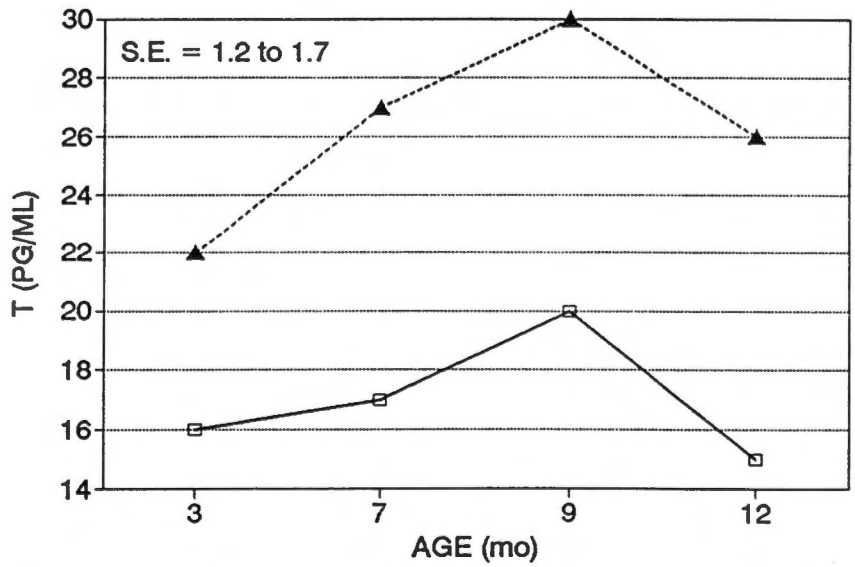

Fig 3. Testosterone secretion in prepuberal heifers

The pattern of estradiol (Fig 4) showed a biphasal increase over the 9 mo period.

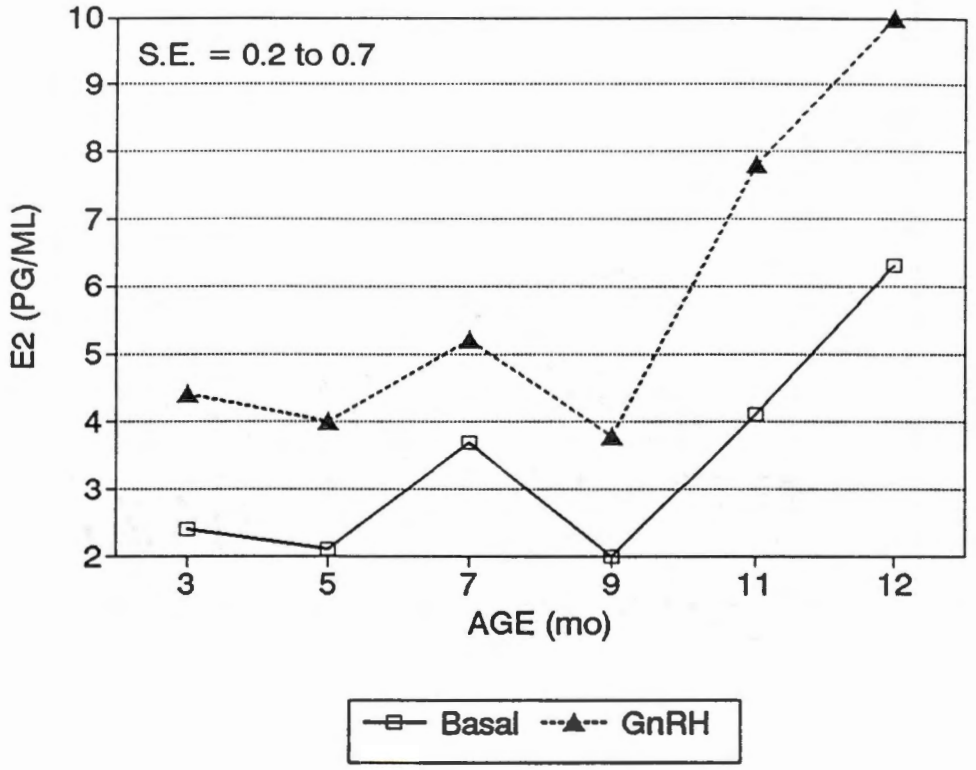

Fig 4. Estradiol secretion in prepuberal heifers

Follicle Counts:

Follicle counts are listed in Table 1 for each heifer. All primary follicles for animal number (ANO) 23 (characterized by having the least number of primary follicles), were located abnormally deep in the ovarian tissue. In the normal ovary, primary follicles are located near the perimeter of the ovary. Heifers with more than 130,000 primary follicles had significantly higher

Table 1. Follicular morphology of twenty 16 mo old Angus heifers

ANO	PRIM[a] (x 10^3)	SEC[b] (x 10^3)	VESICULAR[c] NORMAL[c]	VESICULAR[c] ATR[d]	VESICULAR[c] TOTAL	TOTAL CL
42	501.0	24.0	79	47	126	10
12	340.0	12.5	101	7	108	8
35	287.0	16.5	68	41	109	7
208	283.0	6.6	54	15	69	8
532	273.6	4.8	105	44	149	2
61	266.2	8.8	63	49	112	8
25	260.0	10.4	91	38	129	6
310	256.8	4.8	75	22	97	1
517	229.4	3.1	107	31	138	1
31	225.0	9.0	49	25	74	5
235	201.5	19.5	105	45	149	9
534	195.0	7.8	52	16	68	1
6	191.7	13.5	57	27	84	6
270	177.1	2.3	71	32	103	1
515	156.8	6.4	69	39	108	2
39	156.0	9.0	58	20	78	10
235	151.2	5.6	108	36	144	2
308	150.8	5.2	124	72	196	5
345	137.8	2.6	97	20	117	1
443	132.0	2.2	85	34	119	1
288	116.1	5.4	10	17	27	1
516	108.0	5.4	26	5	31	2
521	95.7	2.9	56	30	86	2
209	86.4	2.4	54	35	89	9
239	85.8	2.6	26	13	39	2
45	83.2	5.2	70	44	114	9
65	81.4	4.4	36	17	53	5
273	75.0	2.5	23	9	32	8
384	60.0	5.0	39	19	58	1
422	52.9	2.3	39	25	64	1
353	48.6	1.8	5	0	5	2
48	46.8	5.2	41	40	81	3
70	45.0	5.0	47	24	71	11
375	37.8	8.1	61	35	96	2
529	37.8	2.1	54	12	66	2
522	16.0	1.0	19	5	24	0
357	4.2	1.0	16	4	20	1
23	3.6	1.8	4	2	6	4

[a]Follicle with one layer of granulosa cells; [b]Follicle with two or more layers of granulosa cells but without a vesicle; [c]Follicle with a fully formed vesicle; [d]Follicle with absent or necrotic granulosa cells; [c]Vesiclular follicles of all sizes

testosterone (7 mo) and Δ_4A (9 & 11 mo) concentrations in their serum than those with a lesser number of follicles ($p<0.01$).

Histologic Correlations:

Primary follicle numbers were positively correlated with number of SEC ($r=.7$, $p<0.01$), normal vesicular ($r=.4$, $p<0.01$) and total vesicular follicles ($r=.5$, $p<0.01$). Secondary follicle numbers were positively associated with normal vesicular follicles ($r=.3$, $p<0.01$), ATR follicles ($r=.4$, $p<0.01$) and total vesicular follicle numbers ($r=.4$, $p<0.01$). Number of normal vesicular follicles were closely associated with number of ATR ($r=.7$, $p<0.01$) and total vesicular follicles ($r=.96$, $p<0.01$).

Correlations between hormones and number of follicles:

A positive correlation was found between number of primary follicles and both basal and GnRH stimulated Δ_4A secretion in heifers at 7 mo of age ($r=.4$, $p<0.01$). Basal and GnRH stimulated secretion of P_4 at 3, 7 & 11 mo exhibited a positive correlation with number of ATR ($r=.4$, $p<0.01$) and total vesicular follicles ($r=.4$, $p<0.01$). Estradiol, both basal and GnRH stimulated, was associated with ATR and total vesicular follicles at 9 mo of age ($r=.5$, $p<0.01$).

DISCUSSION

Results from this study indicate that heifers with the highest number of primary follicles had significantly more androgens in their peripheral blood at 7, 9 and 11 mo ($p<0.01$) than those with reduced numbers of follicles (Table 1). The average number of follicles observed was consistent with Erickson's (1966) previous reports.

Androstenedione concentrations reported here were consistent with Kanchev and Dobson's findings in 1976. No appreciable change in basal Δ_4A levels occurred across the ages (Fig. 1), but the response to GnRH decreased between 5 and 7 mo. Both basal and GnRH progesterone (Fig. 2) and E_2 (Fig. 4) exhibited a significant increase in secretion as puberty approached. Testosterone (Fig. 3) showed an increase in both basal and GnRH secretion at 9 mo of age. This increase in hormonal concentrations just prior to puberty, is probably a consequence of advancement in follicular growth.

Primary and secondary follicle numbers were correlated with normal vesicular, ATR, and total vesicular follicles ($r=.5$, $p<0.01$). This is not surprising since primary follicles are the "pool" from which all other follicles arise. Thus, those heifers with a fewer number of primary follicles (Table 1) also had lower advanced follicular counts.

A definite point of separation occurred between those animals with a high number of vesicular follicles and those

with a lower number. As can be seen in Table 1, this split occurred between those heifers with more or less than 130,000 primary follicles. As the number of primary follicles decreased a corresponding decline in number of growing follicles was noted. This supports the theory that a high number of growing and vesicular follicles is usually associated with a high number of primary follicles.

Heifers with primary follicle numbers of 130,000 or greater had significantly higher Δ_4A at 9 & 11 mo ($p<0.01$), indicating a greater number of growing, ATR and total vesicular follicles. This would be expected since the major source of ovarian Δ_4A is from small vesicular and ATR follicles (Campbell et al., 1990a; Ireland and Roche, 1983; Kruip and Dielman, 1985; McNatty et al., 1985).

In conclusion, circulating levels of androstenedione in the 9-11 mo old heifer are not only an indicator of the number of total vesicular follicles but also a potential indicator of reproductive capacity. Those animals with lower Δ_4A concentrations also had a lower number of primary follicles, ultimately indicating a likelihood of a shortened reproductive life.

LIST OF REFERENCES

Ainsworth, L., B. K. Tsang, B. R. Downey and G. J. Marcus. 1990. The synthesis and actions of steroids and prostaglandins during follicular maturation in the pig. J. Reprod. Fert. Suppl. 40:137.

Cameron, A. W. N., C. M. Oldham, I. J. Fairnie, E. J. Keogh and D. R. Lindsay. 1988. the number of spermatozoa, the number of ovulations per ewe, and immunization against androstenedione affect fertility and prolificacy in sheep. J. Reprod. Fert. 84:43.

Campbell, B. K., G. E. Mann, A. S. McNeilly and D. T. Baird. 1990a. Pulsatile secretion of inhibin, oestradiol and androstenedione by the ovary of the sheep during the oestrous cycle. J. Endocrin. 126:385.

Campbell, B. K., G. E. Mann, A. S. McNeilly and D. T. Baird. 1990b. The pattern of ovarian inhibin, estradiol, and androstenedione secretion during the estrous cycle of the ewe. Endocrinology 127:227.

Coffey, D. S. 1988. Androgen action and the sex accessory tissues. In: The Physiology of Reproduction. E. Knobil and J. D. Neill, Editors-in-Chief. Vol. II, Chpt. 24, pg. 1081. Raven Press, New York.

Connolly, P. B., C. E. Roselli, and J. A. Resko. 1990. Aromatase activity in the adult guinea pig brain is androgen dependent. Biol. Reprod. 43:698.

D'Occhio, M. J. D., D. R. Gifford, R. M. Hoskinson, T. Weatherly and B. P. Setchell. 1988. Gonadotropin secretion and ovarian responses in prepubertal heifers actively immunized against androstenedione and oestradiol-17β. J. Reprod. Fert. 83:159.

Erickson, B. H. 1966. Development and senescence of the postnatal bovine ovary. J. Anim. Sci. 25:800.

Erickson, G. F., D. A. Magoffin, C. A. Dyer and C. Hofeditz. 1985. The ovarian androgen producing cells: A review of structure/function relationships. Endocrine Reviews 6:371.

Fortune, J. E. and W. Hansel. 1985. Concentrations of steroids and gonadotropins in follicular fluid from normal heifers and heifers primed for superovulation. Biol. Reprod. 32:1069.

Griffin, F. E. and S. R. Ojeda. 1988. Textbook of Endocrine Physiology. Oxford University Press. N.Y., N.Y.

Guyton, A. C. 1976. Endocrinology and Reproduction. In: Textbook of Medical Physiology. 5th edition, Chapt. 77. W. B. Saunders Co. Philadelphia, PA.

Handa, R. J. 1987. Effect of estrogen on androgen receptor dynamics in female rat pituitary. Endocrinology 121:84.

Henderson, K. M. and P. Franchimont. 1981. Regulation of inhibin production by bovine ovarian cells in vitro. J. Reprod. Fertil. 63:431.

Hild-Petito, S., N. B. West, R. M. Brenner, and R. L. Stouffer. 1991. Localization of androgen receptor in the follicle and corpus luteum of the primate ovary during the menstrual cycle. Bio. of Repro. 44:561.

Ireland, J. J. and J. F. Roche. 1983. Development of nonovulatory antral follicles in heifers: Changes in steroids in follicular fluid and receptors for gonadotropins. Endocrinology 112:150.

Kanchev, L. N. and H. Dobson. 1976. Plasma concentration of androstenedione during the bovine oestrus cycle. J. Endocrin. 71:351.

Kanchev, L. N., H. Dobson, W. R. Ward and R. J. Fitzpatrick. 1976. Concentration of steroids in bovine peripheral plasma during the oestrous cycle and the effect of betamethasone treatments. J. Reprod. Fert. 48:341.

Kruip, Th. A. M. and S. J. Dielman. 1985. Steroid hormone concentrations in th fluid of bovine follicles relative to size, quality, and stage of the oestrus cycle. Therio. 24:4.

Marden, W. G. R. 1953. The hormone control of ovulation in the calf. J. Anim. Sci. 43:381.

McKinnie, M. R., J. H. Britt and K. L. Esbenshade. 1988. Ovarian function and hormone secretion of gilts actively immunized against androstenedione. J. Anim. Sci. 66:3131.

McNatty, K. P., M. Gibb, C. Dobson, D. C. Thurley and J. K. Findlay. 1981. Changes in the concentration of gonadotrophic and steroidal hormones in the antral fluid of ovarian follicles throughout the oestrous cycle of the sheep. Aust. J. Biol. Sci. 34:67.

Norman, A. W. and G. Litwack. 1987. Hormones. Academic Press, Inc. San Diego, CA.

Philipon, P., A. L. Prieur and M. A. Driancourt. 1989. Alterations in gonadotrophin secretion and ovarian sensitivity to gonadotrophins induced by active immunization against androstenedione. In: Animal Reprod. Science 19:53. Elsevier Science Publishers B. V. , Amsterdam.

Peterson, A. J., R. J. Fairclough and J. F. Smith. 1978. Radioimmunoassay of androstenedione and testosterone in cow plasma at the time of luteolysis and oestrus. J. Reprod. Fert. 52:127.

Prevost, J., A. Belanger, C. Bernard and R. D. Lambert. 1989. Androgen metabolites in bovine follicular fluid. Therio. 31:

Roselli, C. E. 1987. Time course and steroid specificity of aromatase induction in rat hypothalamus-preoptic area. Biol. Reprod. 37:628.

Spicer, L. J. and S. A. Zinn. 1987. Relationship between concentration of cortisol in ovarian follicular fluid and various biochemical markers of follicular differentiation in cyclic and anovulatory cattle. J. Reprod. Fert. 81:221.

Statistical Analysis System User's Guide: Statistics 1985. SAS Institute Inc. Cary, North Carolina.

Statistical Analysis System User's Guide: Language and Procedures 1989. SAS Institute Inc. Cary, North Carolina.

Takeda, H., G. Chodak, S. Mutchnik, T. Nakamoto, and G. Chang. 1990. Immunohistochemical localization of androgen receptors with mono- and polyclonal antibodies to androgen receptor. J. Endocrinology 126:17.

Wise, T. H., D. Caton, W. W. Thatcher, A. Lehrer and M. J. Fields. 1982. Androstenedione, dehydroepiandrosterone and testosterone in ovarian vein plasma and androstenedione in peripheral arterial plasma during the bovine oestrus cycle. J. Reprod. Fert. 66:513.

Wise, T. H. and B. D. Schanbacher. 1983. Reproductive effects of immunizing heifers against androstenedione and oestradiol-17β. J. Reprod. Fert. 69:605.

Wise, T. 1987. Biochemical analysis of bovine follicular fluid: Albumin, total protein, lysosomal enzymes, ions, steroids and ascorbic acid content in relation to follicular size, rank, atresia classification and day of estrous cycle. J. Anim. Sci. 64:1153.

GENERAL SUMMARY

SUMMARY

Two studies were conducted to examine the extent to which utilization of prepuberal hormones could be used as an indicator of reproductive potential in beef heifers.

The first study examined interbreed differences in prepuberal hormonal secretion of three breeds (breed-types) of heifers (14 HP, 14 XB and 28 ANG). Radioimmunoassay's were conducted for LH, FSH, P_4, Δ_4A, T & E_2 on basal and GnRH augmented blood samples taken at 6 ages (3, 5, 7, 9, 11 & 12 mo). The second study examined the relationship between gonadal steroids of 39 heifers and their follicular numbers.

Results from the two studies indicated that age at puberty and ovarian morphology can, to some extent, be predicted by prepuberal hormones. Androgens seemed to play a significant role in identifying heifers with the greatest numbers of primary follicles which ultimately may predispose the heifer to a greater reproductive potential.

Crossbred heifers were significantly heavier in birth, weaning and yearling weights while also having higher basal and GnRH stimulated T levels at all ages and higher Δ_4A from 9-12 mo. The higher androgen levels probably act to increase prepuberal weight in the XB heifers thus accelerating the maturation of the hypothalamus and thereby decreasing age at puberty. Polled Herefords were characterized by numerically higher gonadotropin concentrations, lower E_2 levels and an

increased age at ovulation (only one heifer had apparently ovulated by 12 mo of age). Angus were most distinctive in basal and GnRH stimulated secretion of Δ_4A at 3, 5 and 7 mo, and significantly higher LH and FSH secretion in response to GnRH. Heifers which exhibited early signs of puberty (P_4 >1 ng/ml) had significantly higher basal and GnRH stimulated secretions of E_2 (3 mo) and Δ_4A (9 & 11 mo).

Higher basal T at 7 mo and Δ_4A at 9 and 11 mo were found in those heifers with the greatest number of primary follicles. Thus, around the time of weaning, utilization of androgen concentrations could be a valuable tool in identifying those heifers with high numbers of primary follicles.

In conclusion, Δ_4A, T and E_2 secretion near puberty (7-11 mo) appears to be a useful marker for both early age at puberty and number of primary follicles. And, since number of primary follicles is highly correlated with number of growing and vesicular follicles, androgens could be a useful indicator of the reproductive potential of beef heifers.

APPENDICES

APPENDIX A

BUFFERS AND REAGENTS

Sodium Phosphate Buffers (0.5 M)

1. Monobasic F.W. = 137.99 (hydrous or anhydrous)
   To make up 500 ml:
   Add 34.49 g of Sodium Phosphate (monobasic) to a 500 ml volumetric flask then fill halfway with double distilled (DD) H_2O. Stir until dissolved then fill to appropriate volume with more DD H_2O and stir for an additional 10-15 minutes. Filter the solution with a 0.45 μm filter (a vacuum is required). Store at room temperature. Buffer will keep 4-6 weeks.

2. Dibasic F.W. = 268.07 (hydrous or anhydrous)
   To make up 500 ml:
   Add 67 g of Sodium Phosphate (dibasic) to a 500 ml volumetric flask then fill halfway with DD H_2O. Stir until dissolved then fill to appropriate volume with more DD H_2O, and stir for and additional 10-15 minutes. Filter the solution with a 0.45 μm filter (a vacuum is required). Store at room temperature. Buffer will keep for 4-6 weeks.

Iodination Buffers

LH:

1. 0.05 PBS for Chloramine-T and NaMBS preparation
   combine:
   - 0.4 ml of 0.5 M Sodium phosphate buffer, monobasic
   - 2.1 ml of 0.5 M Sodium phosphate buffer, dibasic
   - 22.5 ml of DD H_2O....bring pH to 7.5

2. 0.05 PBS + 0.1 % BSA eluent solution (100 ml solution)

   combine:
   - 1.6 ml of 0.5 M Sodium phosphate buffer, monobasic
   - 8.4 ml of 0.5 M sodium phosphate buffer, diabasic
   - 100 mg BSA
   - 90 ml of DD H_2O....bring pH to 7.5

3. 0.5 M PBS for:
   a) add to protein just prior to addition of ^{125}I
   b) buffer head on ^{125}I syringe

combine:
- 1.6 ml of 0.5 M monobasic
- 8.4 ml of 0.5 M dibasic
- bring pH to 7.5

4. Chloramine-T (1 $\mu g/\mu l$) for:
   a) catalyst for the reaction

   combine:
   - 5 mg of Chloramine-T
   - 5 ml of 0.05 M PBS (pH 7.5)
   15 μl will be used in iodination procedure

5. Sodium Metabisulfite (2 $\mu g/\mu l$) for:
   a) stops the reaction

   combine:
   - 10 mg Sodium Metabisulfite (NaMBS)
   - 5 ml of 0.05 M PBS (Ph 7.5)
   30 μl will be used in iodination procedure

FSH:

1. 0.05 M PBS + 0.1% BSA eluent solution (100 ml solution)
   combine:
   - 1.9 ml of 0.5 M monobasic
   - 8.1 ml of 0.5 M dibasic
   - 100 mg BSA
   - 90 ml of DD H_2O .... bring pH to 7.4

2. 0.5 M PBS for:
   a) add to protein just prior to the addition of ^{125}I
   b) buffer head on ^{125}I syringe

   combine:
   - 1.9 ml of monobasic
   - 8.1 ml of dibasic
   - bring pH to 7.4

3. 0.25 M PBS for:
   a) used to prepare NaMBS

   combine:
   - 5 ml of 0.5 M PBS (pH 7.4) Buffer 2 above
   - 5 ml of DD H_2O

4. NaMBS for:
   a) to stop reaction

combine:
- 5 mg of NaMBS
- 5 ml of 0.25 M PBS (pH 7.4) Buffer 3 above

then combine: (to get 25 μg/ml)
- 100 μl of NaMBS (1 mg/ml)
- 3.9 ml of 0.25 M PBS (pH 7.4)

200 μl will be used in iodination procedure

5. Potassium iodide (KI) (1 mg/ml)
   a) to bind "free" iodine

   combine:
   - 5 mg of KI
   -5 ml of 0.5 M dibasic

   200 μl will be used in iodination procedure

Reagents for LH and FSH

1. Basic RIA Buffer: Use a volumetric flask. Add material to flask before adding liquid. Fill about halfway with DD H_2O and dissolve materials before completing the mixture. Adjust pH to 7.5 using 1 M, 3 M or 10 M NaOH (base) or 5% sulfosalicylic acid (acid).

Materials	Liter
0.1% BSA	1 g
0.135 M NaCl	7.89 g
0.005 M $NaHCO_3$	0.420 g
0.01% Thimerosal	100 mg
Monobasic 0.5 M	3.2 ml
Dibasic 0.5 M	16.8 ml

2. First Ab Buffer: Use a volumetric flask. Mix using DD H_2O. Adjust pH to 7.5 after mixing solution.

Materials	Liter
0.135 M NaCl	7.89 g
0.005 M $NaHCO_3$	0.420 g
0.05 M (Di) NaEDTA	18.6 g
1:300 NRS	3.3 ml
0.01 Thimerosal	100 mg
Monobasic 0.5 M	1.7 ml
Dibasic 0.5 M	18.3 ml

3. 2% Normal Rabbit Serum (NRS) v/v: Use a volumetric flask. Mix using DD H_2O. Adjust pH to 7.5 after mixing solution.

Materials	Liter
Normal Rabbit Serum	20 ml
0.01% Thimerosal	100 mg
Monobasic 0.5 M	3.8 ml
Dibasic 0.5 M	16.2 ml

4. Polyethylene glycol (PEG): Use a volumetric flask. Mix using DD H_2O. Adjust pH to 7.5 after mixing solution.

Materials	Liter
PEG	50 g
0.01% Thimerosal	100 mg
Monobasic 0.5 M	3.2 ml
Dibasic 0.5 M	16.8 ml

Reagents for P_4, Δ_4A and T RIA

1. Assay buffer: Heat 500 ml of distilled water, add gelatin and stir until dissolved. Add other ingredients except for Na azide and dissolve. Add the rest of the distilled water for a total volume of 1 liter. Add the azide when buffer in near room temperature. Unadjusted pH should be 7.4±0.1.

Materials	Liter
$NaH_2PO_4.H_2O$	2.1 g
Na_2HPO_4 anhydrous	8.75 g
NaCl	8.75 g
Na azide	1.0 g
Gelatin	1 g

2. Dextran coated charcoal (steroid): Heat buffer or allow it to come to room temperature and dissolve Dextran into it. Add Norit A and stir for 30 minutes. Store in refrigerator.

Materials	400 ml
Steroid Buffer	400 ml
Dextran (T-70)	0.25 g
Norit A	2.5 g

Reagents for E_2 RIA

1. Assay Buffer: Make each solution as described below. Use monobasic to balance pH of dibasic to 7.0. Add 1 g of gelatin to 1 liter solution. It will be necessary to heat and stir to get gelatin into solution. This

should not change the pH. Run a blank containing the buffer solution.

Materials	Monobasic	Dibasic
DD H_2O	1 Liter	1 Liter
Na_2PO_4 H_2^O	1.38 g	----
Na_2HPO_4	----	1.42 g
NaCl	8.7 g	8.7 g
Thimerosal	0.1 g	0.1 g

Gelatin - Use 1 g/1 l of solution

2. Dextran coated charcoal (E_2): Heat and stir PBS-gel and Dextran until completely dissolved. Add Norit A and stir for 30 minutes. Store in refrigerator (4°C).

Materials	400 ml
PBS-gel (E_2 buffer)	400 ml
Dextran (T-70)(Pharmacia Co.)	0.2 g
Norit A Charcoal(Sigma)	2.0 g

Stock solution for Steroid Standards (P_4, Δ_4A, T E_2)

1. Weigh out 10 mg (0.01 g) of the steroid (Sigma Chemical Co.). Dissolve in 200 ml of ethanol (ETOH). This yields a concentration of 50 µg steroid/ml.

2. Take 1 ml of solution 1 (above) and add to 49 ml of ETOH. Shake well. Store in air-tight container in freezer. This yields a concentration of 1 µg/ml.

Steroid Standard Solution

Prepare a stock solution which contains the steroid at a 1 µg/ml concentration (see above). Shake vigorously after each dilution.

A. 100 µl of stock solution to 10 ml of buffer. This yields a concentration of 1,000 pg/100 µl.

B. 5 ml of dilution A to 5 ml of buffer = 500 pg/100 µl.

C. 5 ml of dilution B to 5 ml of buffer = 250 pg/100 µl.

D. 4 ml of dilution C to 6 ml of buffer = 100 pg/100 µl.

E. 5 ml of dilution D to 5 ml of buffer = 50 pg/100 µl.

F. 5 ml of dilution E to 5 ml of buffer = 25 pg/100 μl.

G. 4 ml of dilution F to 6 ml of buffer = 10 pg/100 μl.

H. 5 ml of dilution G to 5 ml of buffer = 5 pg/100 μl.

I. 5 ml of dilution H to 5 ml of buffer = 2.5 pg/100 μl.

APPENDIX B

RADIOIODINATION PROCEDURES

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

Materials needed:

12 x 75 culture tube (Baxter)
0.5 M Phosphate buffer (pH 7.5) (PB)
0.05 M Phosphate buffer (pH 7.5) (PB)
Cloramine-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 (Pharmacia Co.)

Proceed in a step-wise addition:

1. 5 μg LER 1072-2 in 25 μl 0.5 M PB
2. Add 500 μCu (5μl) ^{125}I (Amersham Co.)
3. Add 15 μg (15μl) of Chloramine-T
4. Mix gently for 30 seconds
5. Add 60 μg (30 μl) of Sodium Metabisulfite
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 of tracer and salts
8. Elute with 0.05 M PB + 0.1% BSA (1 ml at a time)

FSH: Iodogen Technique (Bolt 1983)
Protein: USDA bFSH-BP3

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 μg/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 culture tube 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 above to 900 μl of chloroform
3. 50 μl of 2 (above) is put in the 12 x 75 culture

tube 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 μCu ^{125}I (Amersham Co.)
3. Agitate 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 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 for LH is 7.5 and for FSH is 7.4.

Isolation Techniques:

Upon loading iodinated material onto the column, isolation of the labelled protein is accomplished by collecting the eluate in 1 ml fractions. A portable Geiger counter is used to monitor radioactivity. 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.

Calculation of specific activity of labelled hormone: (amount of radiation per unit of hormone)

Take 3 μg of fractions collected in tubes 4 & 5 (above). Run in the Gamma counter and take the cpm/3 μg x 1,000 = total counts in 1 μg. Take that value/35,000 (total of counts you want) / 1000 = counts in 1 ml. This is how much you need to dilute to get a working solution. Take that value and subtract 1 for the ml to be used. Test again.

APPENDIX C

RADIOIMMUNOASSAY TECHNIQUES

C-1. Protocol for LH and FSH RIA. Modified Brown (1983) and Bolt & Rollins (1983) respectively.

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	100	100	100
QC	31-32	150	250	100	100
SAMPLE	33-	150	250**	100	100

**Sample volume (sample & buffer) must equal 400 µl. FSH uses 250 µl sample and 150 µl buffer; LH uses 100 µl sample and 300 µl buffer.

C-1. (Continued) After 48 hour incubation add:

TUBE	#	NRS (µl)	2nd Ab (µl)	PEG (µl)
TC	1-3	---	---	---
NSB	4-6	100	100	500
B0	7-9	100	100	500
STANDARDS	10-30	100	100	500
QC	31-32	100	100	500
SAMPLE	33-	100	100	500

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

NSB= Nonspecific binding, amount of tracer not being bound to Ab or absorbed by charcoal

B0= Total percent 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 plasma sample used in all assays to monitor intra- and interassay variation

Samples= Plasma whose hormonal concentration is to be measured

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 100 µl of 1st Ab (for FSH, 200 µl for LH) to all tubes except TC & NSB
5. Add 100 µl tracer to all tubes
6. Vortex all tubes except TC
7. Incubate 48 hours at room temperature
8. Add 100µl Normal Rabbit Serum (NRS) to all tubes except TC
9. Add 100 µl 2nd Ab to all tubes except TC
10. Add 500 µl Polyethylene glycol (PEG) to all tubes except TC
11. Vortex all tubes except TC
12. Incubate 5 hours at room temperature
13. Centrifuge at 1764 x g for 15 minutes.
14. Decant the supernatant into "Radioactive" labeled bottles
15. Let tubes drain upside down for 10 minutes in box labelled "Radioactive" lined with foil, paper towels and kimwipes
16. Count the tubes in a gamma counter
17. Discard gloves, foil, paper towel and kimwipes in a barrel labelled "Radioactive < 90 days"

Appendix A contains detailed recipes for buffers and reagents to be used for LH and FSH RIA.

C-2. Protocol for P_4, Δ_4A and T RIA modified from Jackson et al (1989)

TUBE	BUFFER (µl)	SAMPLE (µl)	Ab (µl)	TRACER (µl)	CHARCOAL (µl)
TC	650	---	---	100	---
NSB	450	---	---	100	200
B0	250	---	200	100	200
STANDARDS	150	100	200	100	200
SAMPLE	250	200	200	100	200

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

1. Add 200 µl of unknown sample to 13 x 100 tubes

(Baxter)
2. Add 1.5 ml Benzene (Fisher Scientific)
3. Vortex 3 times, 1 minute each time, with 5 minute intervals using Multitube Vortexer S/P (Baxter)
4. Allow sample to sit for 15 minutes
5. Aspirate 200 µl of serum from bottom of tube and discard
6. Decant supernatant into 12 x 75 tube
7. Do not allow the serum to mix with the supernatant
8. Place tube in a water bath (48°C)
9. Evaporate benzene with nitrogen
10. When completely dry, add 250 µl steroid buffer
11. Add buffer to each of the 20 assay tubes to be used in for the standard curve (TC, NSB, B0 + 7 standards - each is in duplicate)
12. Add 100 µl of appropriate standard solution to tubes
13. Add 200 µl Ab to all tubes except TC and NSB
14. Add 100 µl tracer to all tubes
15. Vortex on for 1 minute
16. Incubate for 16 h at 4°C
17. Add 200 µl chilled Dextran coated charcoal to all tubes except TC and vortex 1 minute
18. Incubate for 10 minutes at 4°C
19. Centrifuge at 1764 x g for 13 minutes
20. Decant supernatant into plastic scintillation vials
21. Discard charcoaled tubes into barrel labelled "Radioactive > 90 days"
22. Add 5 ml Econofluro-2 scintillation fluid (Dupont)
23. Apply lids tightly and shake vigorously
24. Count vials for 1 minute in a liquid scintillation counter
25. Discard gloves in barrel labelled "Radioactive > 90 days"

Appendix A contains details of steroid buffer, dextran coated charcoal and standard solution recipes.

C-3 Protocol for E_2 RIA modified from Britt (1990)

TUBE	BUFFER (µl)	SAMPLE (µl)	Ab (µl)	TRACER (µl)	CHARCOAL (µl)
TC	950	---	---	100	---
NSB	450	---	---	100	300
B0	250	---	200	100	300
STANDARDS	150	100	200	100	300
SAMPLE	250	200	200	100	300

Proceed in a step-wise addition: (wear gloves at all times)
Steps 1-10 are exactly the same as for the other steroid RIA protocols (listed above)

11. Add buffer to each of the 30 assay tubes to be used for the standard curve (TC, NSB, B0 + 7 standards - each in triplicates)
12. Add 100 µl of standard solution to appropriate tube
13. Add 200 µl Ab to all tubes except TC and NSB
14. Incubate 16 hours at 4°C
15. Add 100 µl tracer to all tubes
16. Vortex on for 1 minute
17. Incubate 5 hours at 4°C
18. Add 300 µl chilled Dextran coated charcoal to all tubes except TC and vortex 1 minute
19. Incubate 10 minutes at 4°C
20. Centrifuge at 1764 x g for 13 minutes.
21. Decant supernatant into 12 x 75 tubes
22. Discard charcoaled tube in barrel labelled "Radioactive < 90 days"
23. Count tubes for 1 minute in a gamma counter
24. Discard gloves in barrel labelled "Radioactive < 90 days"

Appendix A contains details of steroid buffer, dextran coated charcoal and standard solution recipes.

VITA

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