

/ EVIDENCE FOR A FOLLICLE STIMULATING HORMONE
BINDING INHIBITOR (FSH-BI) /

by

MARY ANN GILSDORF

B.S., Kansas State University, 1983



A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree


MASTER OF SCIENCE

Department of Animal Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1984

Approved by:


Major Professor

LD
2668
.T4
1984
G53
c. 2

TABLE OF CONTENTS

I. EVIDENCE FOR A BOVINE FOLLICLE STIMULATING HORMONE BINDING INHIBITOR (FSH-BI)

	Page
LITERATURE REVIEW.....	1
LITERATURE CITED IN LITERATURE REVIEW.....	4
INTRODUCTION.....	5
MATERIALS AND METHODS.....	6
RESULTS AND DISCUSSION.....	8
LITERATURE CITED.....	19

II. CHANGES IN FSH-BI CONCENTRATION IN FOLLICULAR FLUID DURING THE ESTROUS CYCLE OF THE BEEF HEIFER

INTRODUCTION.....	15
MATERIALS AND METHODS.....	16
RESULTS.....	17
LITERATURE CITED.....	19

LIST OF TABLES

Table		Page
1	EFFECT OF BOVINE FOLLICULAR FLUID ON OVARIAN WEIGHT OF PREPUBERAL RATS GIVEN bFSH AND hCG.....	10
2	COMPARISON OF NONEXTRACTED AND CHARCOAL EXTRACTED FOLLICULAR FLUID (FF) ON OVARIAN WEIGHT OF PREPUBERAL RATS GIVEN bFSH AND hCG.....	11
3	COMPARISON OF PHYSIOLOGICAL SALINE AND BOVINE BLOOD SERUM ON OVARIAN WEIGHT OF PREPUBERAL RATS GIVEN bFSH AND hCG.....	11
4	EFFECT OF VARYING LEVELS OF BOVINE FOLLICULAR FLUID (FF) ON OVARIAN WEIGHT OF PREPUBERAL RATS PRIMED WITH bFSH AND hCG.....	14

LITERATURE REVIEW

Proteins that regulate follicular growth and response to gonadotropins have recently been found in bovine follicular fluid (FF). A clearer understanding of those regulators could result in increased reproductive efficiency. Recently, researchers have investigated the biochemical makeup of the protein regulators and their mechanisms affecting folliculogenesis. However, this area of study is new and the physiological mechanisms proposed are speculative. Since this area of research is relatively new, literature pertaining specifically to gonadotropin inhibitors at the ovarian level is not extensive. Thus, this review is intended to point out the specific research that has led to the physiological studies of biologically active factors in follicular fluid that inhibit ovarian follicular response to follicle stimulating hormone (FSH), and to determine if those factors have a physiological role in controlling the estrous cycle.

Ovarian follicles do not respond equally to similar cyclic hormonal conditions which suggests that individual follicles have their own regulators (Richards and Midgley, 1976). Maturation of antral follicles appears to be dependent on FSH and maturing follicles show an increase in receptors for luteinizing hormone (LH) in granulosa cells, an elevated secretion of estradiol, and a high ratio of estrogens to androgens. Atresia in antral follicles is associated with a decrease in granulosa cell receptors for both LH and FSH with smaller estrogen to androgen ratios (Ireland and Roche, 1983; Sluss et al., 1983). Some of these follicular changes may be internally regulated while others may be externally regulated. The presence of inhibin in FF and its ability to suppress FSH secretion is well documented. Injections of FF lowered serum FSH levels in ovariectomized heifers (Kiracofe et al., 1983) intact bulls (McGowan et al., 1984) and adult male

rats (Sheth et al., 1983). Sato et al. (1982) injected FF in unilaterally ovariectomized mice and decreased serum FSH levels and compensatory ovarian hypertrophy. These researchers cited inhibin as the proteinaceous compound responsible for their experimental results. However, not all follicular changes can be explained by the level of FSH secretion. Sluss and Reichert (1983) found that FF inhibited attachment of FSH to its binding sites in both granulosa cells and homogenates of calf testis. This factor does not appear to be inhibin. The molecular weight of inhibin as determined by gel electrophoresis is around 19,000 (Sheth et al., 1983) while the fractionated portion of FF that suppressed FSH binding to its receptor sites had a molecular weight of less than 5000 (Reichert et al., 1982).

Isolation of an FSH binding inhibitor (FSH-BI) has been accomplished and determined to be a protein after fractionation, heat treatment, charcoal extraction and exposure to trypsin (Sluss et al., 1983; Sato et al., 1980; Kalla and Zarabi, 1984).

Concentration of FSH-BI is highest in pools of fluid from cows in the luteal phase of their estrous cycle with small luteal follicles (<10mm) having a higher concentration than larger luteal follicles (Sluss et al., 1983). There is a high correlation ($r=.97$) between FSH-BI concentration and the relative degree of follicular atresia (Sluss and Reichert, 1983). Immunohistochemical studies carried out by Sato et al. (1982) indicate the inhibitor is located in the granulosa cells.

Other evidence supports a physiological role for FF proteins in the regulation of reproductive function. Dizerga et al. (1983) referred to an inhibition of aromatase activity and interference with folliculogenesis in monkeys following FF treatment. Miller et al. (1979) reported injections of steroid extracted FF interrupted the sequence of normal follicular development in sheep and cattle.

These data are consistent with those of Sato et al. (1982) and indicate that follicles containing the inhibitor may be prevented from progressing further in development.

LITERATURE CITED

- Dizerga, G.S., Campeau, J.D., Nakamura, R.M., Ujita, E.L., Lobo, R., and Marrs, R.P. Activity of a human follicular fluid protein(s) in spontaneous and induced ovarian cycles, *J. Clin. Endocrinol. Metab.* 57: 838-846 (1983).
- Ireland, J.J., and Roche, J.F. Growth and differentiation of large antral follicles after spontaneous luteolysis in heifers: change in concentration of hormones in follicular fluid and specific binding of gonadotropins to follicles, *J. Anim. Sci.* 57: 157-167 (1983).
- Kalla, N.R., and Zarabi, S. Studies of Follicle Stimulating Hormone Receptor Binding Inhibitor (FSH-RBI), *Horm. Metab. Res.* 16: 212-213 (1984).
- Kiracofe, G.H., Ramirez-Godinez, J.A., McGowan, R.D., and Bolt, D.J. Reduction of serum FSH in ovariectomized heifers with bovine follicle fluid, *J. Anim. Sci.* 57 (Suppl 1): 350 (1983). (Abstract).
- Miller, K.F., Critser, J.K., Rowe, R.F., and Ginther, O.J. Ovarian effects of bovine follicular fluid treatment in sheep and cattle, *Biol. Reprod.* 21: 537-544 (1979).
- McGowan, R.D., Kiracofe, G.H., Middleton, C.D., and Bolt, D.J. Sexual behavior and semen production of bulls after reduction of FSH by bovine follicular fluid, *J. Anim. Sci.* 59 (Suppl 1): 119 (1984). (Abstract).
- Reichert, L.E., Jr., Sanzo, M.A., Fletcher, P.W., Dias, J.A., and Lee, C.Y. Properties of Follicle Stimulating Hormone Binding Inhibitor found in physiological fluids, *Adv. Exp. Med. Biol.* 147: 135-144 (1982).
- Richards, J.S., and Midgley, A.R. Protein hormone action: a key to understanding ovarian follicular and luteal cell development, *Biol. Reprod.* 14: 82 (1976)
- Sato, E., Ishibashi, T., and Iritani, A. Effect of inhibin-like substance isolated from porcine follicular fluid on the Follicle Stimulating Hormone (FSH) level in mouse serum and on FSH binding to porcine granulosa cells, *Fert. Steril.* 34: 55-57 (1980).
- Sato, E., Ishibashi, T., and Iritani, A. Purification and action sites of a Follicle Stimulating Hormone Inhibitor from bovine follicular fluid, *J. Anim. Sci.* 55: 873-877 (1982).
- Sheth, A.R., Sheth, P.R., and Roy, R. Interaction of thyrotrophin releasing hormone with inhibin in male rats, *J. Endocr.* 98: 1-6 (1983).
- Sluss, P.M., and Reichert, L.E., Jr. Presence of bacteria in porcine follicular fluid and their ability to generate an inhibitor of Follicle Stimulating Hormone Binding to receptor, *Biol. Reprod.* 29: 335-341 (1983).
- Sluss, P.M., Fletcher, P.W., and Reichert, L.E., Jr. Inhibition of ¹²⁵I-human follicle stimulating hormone binding to receptor by a low molecular weight fraction of bovine follicular fluid: inhibitor concentration is related to biochemical parameters of follicular development, *Biol. Reprod.* 29: 1105-1113 (1983).

EVIDENCE FOR A BOVINE FOLLICLE STIMULATING HORMONE
BINDING INHIBITOR (FSH-BI)

INTRODUCTION

Improved reproductive efficiency in the bovine, in many cases, is dependent on a clearer understanding of ovarian follicular growth and maturation. Some factors have been identified in bovine follicular fluid (FF) that might control follicular maturation. Follicular fluid contains a factor capable of delaying estrus in prostaglandin treated cows (1) and suppressing follicle stimulating hormone (FSH) in serum of ovariectomized heifers (2) and intact bulls (3). These effects have been attributed to a protein called inhibin, which influences pituitary secretion of FSH. In addition to inhibin, a low molecular weight protein (<6,000) has been isolated from bovine FF which inhibits in vitro binding of FSH in homogenates of calf testis (4). This FSH binding inhibitor (FSH-BI) prevented compensatory ovarian hypertrophy in mice and inhibited binding of FSH to granulosa cells in vitro (5). Concentration of the FSH-BI appears to be correlated with the physiological state of the follicle (4).

Since bovine FSH-BI could be a major factor in regulating ovarian follicular maturation, it is essential that further evidence for its existence be shown. Data are available which suggest a non-steroidal factor in FF interferes with pituitary control of follicular growth, but little information exists for one that works at the ovarian level. The present experiments tested the ability of bovine ovarian FF to affect the ovarian weights of prepuberal rats given bFSH and human chorionic gonadotropin (hCG).

MATERIALS AND METHODS

Collection of Follicular Fluid

Reproductive tracts were obtained from mature cows as they were slaughtered at Jack Polen Beef Packers, Kansas City, KS. The horns of each tract were incised and pregnancy determined by the presence of a conceptus. If no conceptus was present, stage of the estrous cycle was determined by the presence or absence of a corpus luteum (CL) that appeared functional. Ovaries with functional CL were classified luteal while ovaries with atretic CL were classified follicular. Follicular diameters were measured with a ruler pressed against the follicle. Those with an antrum greater than 8mm in diameter were classified large and those 8mm or less were classified small.

Fluid from ovarian follicles was aspirated using a syringe and an 18 gauge hypodermic needle. Fluid was then divided into six pools according to classification of the reproductive tract and follicles: pregnant large, pregnant small, follicular large, follicular small, luteal large, and luteal small. The pools of fluid were kept on ice until frozen (-20C) approximately 8 hours later. The FF was thawed just before use and refrigerated at approximately 5C during the injection period.

Blood Serum, Hormones and Animals

Serum used for injection in all trials was obtained from four beef heifers bled by jugular venipuncture. Purified bovine FSH (USDA-b-FSH-B-1) was generously provided by Dr. Douglas Bolt, USDA, Beltsville, MD. The hCG was a commercial preparation (Ayerst Laboratories, New York, NY). The bFSH and hCG were dissolved in physiological saline and mixed to provide the appropriate dose in a .2ml injection. Twenty-two day old female Sprague Dawley rats were obtained from Hilltop Laboratories, Scottdale, PA. The rats were kept in an environmentally controlled room with 12 hours light and 12 hours dark with feed and water available ad libitum. All rats were 23 days old at the start of each trial and 26 days old when sacrificed.

Trial 1

In the first trial, 48 rats were utilized in a Steelman-Pohley bioassay (6). Four levels of FSH were administered. Each level was given in 9 sc injections with rats receiving a total of either 0, 30, 60, or 120ug bFSH/1.8ml. A total of 20IU of hCG was administered along with the bFSH in the 9 injections. The hormone combination was given in .2ml per injection. Twelve rats were assigned to each of the 4 hormone levels with half of the rats in each hormone level receiving pooled (large and small) bovine luteal FF in a .3ml sc injection at each of the 9 injection periods. The other half received a sc injection of .3ml bovine blood serum. Therefore, each rat was injected twice at each of the 9 injection periods. All pools of FF were assayed for FSH-BI and results are reported in another paper (4). Luteal phase FF was used in this study because FSH-BI activity was highest in our luteal fluid pools. Rats were injected every 8 hours for 3 days. Eight hours after the last injection, the rats were killed with chloroform. Weights were taken on each animal, then their reproductive tract was removed. Using a dissecting scope with 6x magnification, the uterus and ovaries were trimmed of adnexal tissues with care being taken to keep the ovaries moist. Weights were then obtained for the paired ovaries and for the uterus using a torsion balance.

Trial 2

The second trial was a repetition of the first except for the addition of a 90ug bFSH group. This trial utilized 60 rats in 5 hormone treatment levels: 0, 30, 60, 90, and 120ug bFSH with a total of 20IU hCG in each level. Weights of rats and paired ovarian weights were recorded.

Trial 3

The third trial utilized 24 rats, equally divided into 4 groups. Each group received the same level of hormone (30ug bFSH plus 20IU hCG) in 9 sc injections of .2ml. In addition, group 1 received non-extracted luteal FF, group 2 received charcoal

extracted FF, group 3 received bovine blood serum and group 4 received saline. All blood serum and FF injections were in .3ml at each of the 9 injection periods. The FF was extracted with charcoal by mixing 50mg of charcoal per ml of FF, stirring for 1 hour at room temperature, centrifuging for 1 hour at 9,000xG at 5C, then filtering the supernatant through .45um filter paper. Weights of rats and paired ovarian weights were taken 8 hours following the last injection period.

Trial 4

Ninety-six rats were divided into 2 hormone treatments with 4 sub-treatments in each group. The 2 hormone levels used were 30ug bFSH plus 20IU hCG and 60ug bFSH plus 20IU hCG. Within the 30ug bFSH group, 4 treatments were administered: .15ml FF, .3ml FF, .45ml FF and .3ml bovine blood serum. In the 60ug bFSH group the four treatments were: .3ml FF, .45ml FF, .6ml FF and .3ml bovine blood serum. All FF used was from a pool of luteal phase ovarian follicles. The injection schedule and data collection were as described in trial 1.

Statistics

Data from Trial 1 and 2 were pooled and analyzed using Statistical Analysis Systems (SAS) two-way analysis of variance. Trial 3 data were analyzed using SAS one-way analysis of variance and Trial 4 using one-way analysis of covariance (7).

RESULTS AND DISCUSSION

Trials 1 and 2

Overall, rats injected with non-extracted bovine luteal FF had lighter ovarian weights compared to those injected with bovine blood serum ($P < .006$). Although ovarian weights of FF treated rats were consistently lighter than those from serum injected rats, a significant treatment effect was observed only within rats treated with 30 ug bFSH (Table 1). In the original Steelman-Pohley bioassay, levels up to 200ug

bFSH plus 20IU hCG resulted in a linear increase in ovarian weight. Our results show a linear response in ovarian weight until the 90ug bFSH level, where it increased rapidly. This erratic response may have been due to increased potency of bFSH which is currently available compared with that utilized in the original Steelman-Pohley assay. Uterine weights were not significantly different between levels. Uterine weights were taken as an indicator of estrogen stimulation (8). However, since average uterine weights were not different (serum = 156.5 ± 20.25 mg, FF = 155.5 ± 35.39 mg), and appeared to be maximally stimulated by the bFSH-hCG treatment, they were not taken in subsequent trials.

Trial 3

Non-extracted bovine luteal FF was not significantly different than charcoal extracted bovine FF in its ability to suppress ovarian weight ($P < .39$) indicating that steroid hormones present in FF were not responsible for the ovarian weight suppression (Table 2). This agrees with data from Miller et al. attributing FSH binding activity to a non-steroidal substance. It has been shown that charcoal extraction does remove all radioimmunoassayable estrogens (2). Although not significantly different, ovarian weights from rats injected with charcoal extracted FF tended to be lower than those from nonextracted FF treated rats. This could be due to the extraction procedure taking out granulosa cells and other cellular debris, thus concentrating FSH-BI in the extracted FF compared with the nonextracted FF.

Bovine blood serum and physiological saline did not differ in their affect on ovarian weights ($P < .5$) indicating that the normal constituents of serum were not contributing to alterations in ovarian weights (Table 3). Previous reports that progesterone suppressed compensatory ovarian hypertrophy of unilaterally ovariectomized rats (9) and the well-known estrogen suppression of bFSH prompted an investigation of the affect of hormone levels in our bovine blood serum on ovarian

Table 1

EFFECT OF BOVINE FOLLICULAR FLUID ON
OVARIAN WEIGHT OF PREPUBERAL RATS
GIVEN bFSH AND hCG

FSH(ug/rat) ^a	TREATMENT	OVARIAN WT(mg) ^b	P VALUE
0	S	49.08	0.47
0	FF	44.33	
30	S	67.75	.01
30	FF	50.75	
60	S	73.33	0.18
60	FF	64.50	
90	S	79.12	0.34
90	FF	70.29	
120	S	104.75	0.44
120	FF	99.67	

^a Each rat also received 20 IU hCG.

^b Least square means \pm standard error of 4.6mg for 12 rats in each group except the 90ug FSH groups which had 6 rats each.

TABLE 2

COMPARISON OF NONEXTRACTED AND CHARCOAL EXTRACTED FOLLICULAR FLUID (FF) ON OVARIAN WEIGHT OF PREPUBERAL RATS GIVEN bFSH AND hCG

TYPE OF FF	OVARIAN WEIGHT ^a	P VALUE
NONEXTRACTED	59.4	0.39
EXTRACTED	52.6	

^a Least square mean \pm standard error of 5.4mg for 6 rats in each group.

TABLE 3

COMPARISON OF PHYSIOLOGICAL SALINE AND BOVINE BLOOD SERUM ON OVARIAN WEIGHT OF PREPUBERAL RATS GIVEN bFSH AND hCG

TREATMENT	OVARIAN WEIGHT ^a	P VALUE
SALINE	61.1	0.50
SERUM	58.9	

^a Least square means \pm standard error of 5.4mg for 6 rats in each group..

weights.

Trial 4

Treatment with FF, at both levels of bFSH, significantly suppressed ovarian weights below that of serum controls (Table 4). In the 30ug bFSH level, ovarian weights were the same for both .3ml and .45ml FF injections (47mg). In Trial 1 and 2, hCG alone increased ovarian weight to 46.7mg suggesting that maximum suppression of ovarian weight was reached by injections of .3ml and .45ml FF. Although not significant between levels of FF, increasing doses of FF tended to result in lighter subsequent ovarian weights. These data suggest that FSH-BI could be competing with FSH for binding sites in the granulosa cells. By increasing the number of FSH-BI molecules, more FSH is displaced and ovarian follicular growth is decreased. Variations of ovarian weights between trials, given the same hormone and FF treatment, are most likely due to different concentrations of the FSH-BI in particular pools of fluid.

There appears to be little doubt that FF contains an FSH-BI that can decrease the biological effectiveness of circulating FSH. Several modes of action of the FSH-BI have been proposed. One such method is by an inhibition of aromatase activity, thereby reducing levels of estradiol and interfering with folliculogenesis (10). In Trial 1, uterine weights were not affected by FF injections, therefore, it would appear that FF had no dramatic effect on estradiol activity.

A second method proposed is that the FSH-BI protein molecule inhibits binding of FSH to its receptor site (4,11). Our data supports and extends this theory and suggests that binding may be competitive in nature.

It has been suggested that secretion of inhibin by granulosa cells is suppressed as ovulation approaches (12). This suppression brings about a wave of FSH that might be responsible for inducing maturation of a new set of follicles. We propose that FSH-BI plays a role in tying up FSH binding sites so that existing follicles become atretic and

few follicles are allowed to grow and mature. Inhibin may also be suppressed by this mechanism. Additional information is needed on the concentration of FSH-BI in individual follicles throughout the estrous cycle to test this hypothesis.

TABLE 4
EFFECT OF VARYING LEVELS OF BOVINE FOLLICULAR
FLUID (FF) ON OVARIAN WEIGHTS OF PREPUBERAL RATS
PRIMED WITH bFSH AND hCG

FSH(ug/rat) ^a	FF(ml) ^b	OVARIAN WT(mg) ^c	P VALUE ^d
30	0.0	68.5	-----
	0.15	53.8	0.006
	0.3	47.3	0.0001
	0.45	47.6	0.0001
60	0.0	78.3	-----
	0.3	60.3	0.0009
	0.45	58.7	0.0003
	0.6	52.4	0.0001

^a Each rat also received 20IU hCG.

^b The zero group received .3ml bovine blood serum. Numbers represent the amount of FF given at each of the 9 injection periods.

^c Least square means \pm standard error of 3.7mg for 12 rats in each group.

^d Different than serum treatment within the respective FSH level.

CHANGES IN FSH-BI CONCENTRATION IN FOLLICULAR FLUID DURING THE ESTROUS CYCLE OF THE BEEF HEIFER

INTRODUCTION

Recently, the role of proteins in follicular fluid (FF) has been intensely investigated. Biochemical and histochemical studies have eluded to the existence of an FSH-BI that works at the ovarian level in granulosa cells (4, 11). Suppression of utilization of follicle stimulating hormone (FSH) by the ovary has also been attributed to the proteinaceous FSH-binding inhibitor (FSH-BI) (13). However, all the data accumulated to date was derived from pools of FF. Data evaluating concentrations of FSH-BI in individual follicles is not available.

Follicular maturation is associated with an increased concentration of receptors for luteinizing hormone (LH) in granulosa cells and a dramatic increase in the ratio of estrogens to androgens in FF. Atresia in antral follicles corresponds with a decrease in the number of granulosa cell receptors for both LH and FSH, with a decrease in the estrogen to androgen ratio (4).

As follicles near ovulation, estrogen active (E-A) and estrogen inactive (E-I) follicles appear to be equally distributed. In E-A follicles, binding of FSH decreases but granulosa cell numbers increase (14). Within the follicle, FSH-BI could play a role in regulation of the estrous cycle. The purpose of this study was to measure FSH-BI concentrations in FF of individual follicles during the estrous cycle of the heifer, particularly just before and after the ovulatory release of LH and ovulation.

MATERIALS AND METHODS

Forty three 18-month old crossbred heifers housed in dry lot at the Kansas State University Beef Research Unit were visually checked for standing estrus twice daily. Only heifers showing two overt estrous periods 18-22 days apart were selected for the experiment. An intramuscular injection of 30mg of prostaglandin (dinoprost tromethamine; Lutalyse, The Upjohn Co., Kalamazoo, MI) was administered to all heifers in an attempt to synchronize estrus. On day 8 of a subsequent cycle (estrus = day 0), heifers were assigned to one of five groups: (1) ovariectomy (OVX) on day 8, (2) largest follicle marked with charcoal (injected in stroma around follicle) on day 8 then OVX on day 11, (3) prostaglandin injection on day 8 and if no estrus OVX 42 hours later, (4) prostaglandin injection on day 8 then OVX 12 hours after the subsequent standing estrus, and (5) prostaglandin injection on day 8 then OVX 4 days after the subsequent estrus. Immediately after surgical removal of ovaries, follicles were measured two-dimensionally and their fluid aspirated with a syringe and 20 gauge needle. Fluid from follicles larger than 5mm in diameter were identified by size and stored separately while fluid from smaller follicles was pooled. A tissue sample from the wall of the largest follicle of each heifer was removed and stored in Bouins solution. All corpora lutea (CL) were examined and if an antrum was present it was measured and the fluid aspirated and stored separately. All FF samples were frozen at -20C until analysis.

One blood sample was taken by jugular venipuncture before each surgery from the five heifers in group 1 and from the 5 heifers in group 2. All heifers in group 3, 4, and 5 were injected intramuscularly with 30mg of lutalyse on day 8 and were observed for estrus every 6 hours until OVX. No signs of estrus were observed before OVX at 42 hours in the five heifers in group 3. Blood was collected just before prostaglandin injection and then at 12, 24, 36 and 42 hours

after prostaglandin.

Six heifers in group 4 were OVX approximately 12 hours after standing estrus. Blood was collected immediately before the prostaglandin injection, at 12, 24 and 36 hours after prostaglandin then every 6 hours until OVX.

Five heifers in group 5 were OVX 4 days after estrus. Blood collection was the same as in group 4 except samples were taken once daily after estrus until OVX. All blood samples were centrifuged within 24 hours of collection at 2500xG for 20 minutes and the serum filtered off and stored by heifer number and collection time. Blood serum from all animals was frozen and stored at -20C until radioimmunoassayed for FSH and LH by Dr. Douglas Bolt, USDA, Beltsville, MD. The frozen FF and follicle wall samples were shipped to Drs. Leo Reichert and Pat Sluss in the Albany Medical School, Albany, NY for determination of FSH-BI concentration and estrogen:androgen ratios.

Results

Analysis of concentrations of LH in serum indicated that no heifers in group 1, 2, or 3 had ovulatory surges of LH before OVX. Four of the 6 heifers in group 4 had LH surges before OVX, but non had ovulated at OVX. All of the heifers in group 5 had an ovulatory surge and had ovulated before OVX.

A dominant follicle was present in all day 8 OVX heifers along with a well-formed CL. The largest follicle in all heifers in group 2 was positioned next to the functional CL with no apparent change in size over the next 3 days. Concentrations of FSH in the serum of those heifers remained unchanged from day 8 to day 11 and ranged from 11 to 22ng/ml. Perhaps circulating levels of FSH of this magnitude can keep follicles from decreasing in size.

Heifers OVX in group 3 had an easily discernable dominant follicle, as did heifers in group 4. In addition, group 4 heifers had fewer follicles over 6mm than group 3. It would seem that as ovulation approaches, the dominant follicle is the only one to increase in size and fluid content while the others regress. This agrees with findings by Ireland and Roche (14).

Heifers in group 5 (OVX 4 days after estrus) had many antral follicles present on their ovaries. All had a functional new CL but the majority of the largest follicles were contralateral to the CL which was different than the observation of follicles at day 8.

In heifers in which two prostaglandin injections were used to synchronize their estrus and day 8 injection, respectively, the majority had a fluid filled antrum in their CL. Analysis of this and all the FF gathered from all stages in the heifers cycles is currently being carried out. Concentration of FSH-BI will be correlated between stages of the cycle (Groups 1, 2, and 5) and before and after the LH surge (Groups 3 and 4). Follicular wall samples will be observed histologically for granulosa cell numbers which will also be correlated to stage of the cycle and FSH-BI.

ACKNOWLEDGMENTS

I wish to express my deepest love and gratitude to my parents, Mary and Kenny Gilsdorf for their neverending support of my endeavours. If I can only accomplish as much in life and do it as well as they have I will be satisfied.

I thank my brothers and sisters for all the help and support they have given me throughout my academic career.

The companionship and confidence gained from special friends here at K-State is something I will never forget. Without these people, my years at K-State would not be as meaningful or rewarding.

Special thanks go to Dr. Guy Kiracofe and the 'Repro Team': Tom, Carroll, Reggie, Nash, and Jin. My reasearch could not have been carried out without their help and fortitude. I also extend thanks to Drs. Ernie Minton and Larry Corah for their assistance on my committee.

LITERATURE CITED

1. Miller, K.F., Critser, J.K., Rowe, R.F., and Ginther, O.J. Ovarian effects of bovine follicular fluid treatment in sheep and cattle, *Biol. Reprod.* 21: 537-544 (1979).
2. Kiracofe, G.H., Ramirez-Godinez, J.A., McGowan, R.D., and Bolt, D.J. Reduction of serum FSH in ovariectomized heifers with bovine follicle fluid. *J. Anim. Sci.* 57 (Suppl 1): 350 (1983). (Abstract).
3. McGowan, R.D., Kiracofe, G.H., Middleton, C.D., and Bolt, D.J. Sexual behavior and semen production of bulls after reduction of FSH by bovine follicle fluid. *J. Anim. Sci.* 59 (Suppl 1): 119 (1984). (Abstract).
4. Sluss, P.M., Fletcher, P.W., and Reichert, L.E., Jr. Inhibition of ¹²⁵I-human follicle stimulating hormone binding to receptor by a low molecular weight fraction of bovine follicular fluid: inhibitor concentration is related to biochemical parameters of follicular development, *Biol. Reprod.* 29: 1105-1113 (1983).
5. Sato, E., Ishibashi, T., and Iritani, A. Effect of inhibin-like substance isolated from porcine follicular fluid on the follicle-stimulating hormone (FSH) level in mouse serum and on FSH binding to porcine granulosa cells, *Fert. Steril.* 34: 55-57 (1980).
6. Steelman, S.L., and Pohley, F.M. Assay of the follicle stimulating hormone based on the augmentation with human chorionic gonadotropin, *Endocrin.* 53: 604-616 (1953).
7. SAS. SAS User's Guide. Statistical Analysis System Institute, Inc., Cary, N.C., (1982).
8. Astwood, E.B. A six hour assay for the quantitative determination of estrogen. *Endocr.* 23: 25 (1938).
9. Benson, B., Sorrentino, S., and Evans, J.S. Increase in serum FSH following unilateral ovariectomy in the rat, *Endocr.* 84: 369 (1969).
10. Dizerga, G.S., Campeau, J.D., Nakamura, R. M., Ugita, E.L., Lobo R., and Marrs, R.P. Activity of a human follicular fluid protein(s) in spontaneous and induced ovarian cycles, *J. Clin. Endocrinol. Metab.* 57: 838-846 (1983).
11. Sato, D., Ishibashi, T., and Iritani, A. Purification and action sites of a follicle stimulating hormone inhibitor from bovine follicular fluid, *J. Anim. Sci.* 55: 873-877 (1982).
12. Cummins, L.J., O'Shea, T. Bindon, B.M., Lee, V.W., and Findlay, J.K. Ovarian inhibin content and sensitivity to inhibin in Booroola and control strain Merino ewe. *J. Reprod. Fert.* 67: 1-7 (1983).

13. Gilsdorf, M.A., Kiracofe, G.H., Evidence for a bovine FSH-binding inhibitor, *J. Anim. Sci.* 59 (Suppl 1): 78 (1984). (Abstract).
14. Ireland, J.J., and Roche, J.F. Growth and differentiation of large antral follicles after spontaneous luteolysis in heifers: Change in concentration of hormone in follicular fluid and specific binding of gonadotropins to follicles, *J. Anim. Sci.* 57: 157-167 (1983).

EVIDENCE FOR A FOLLICLE STIMULATING HORMONE
BINDING INHIBITOR (FSH-BI)

by

MARY ANN GILSDORF

B.S., Kansas State University, 1983

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1984

Follicular fluid (FF) was collected from ovarian follicles of nonpregnant slaughtered cows. Fluid was aspirated from follicles on ovaries classified as luteal by the gross appearance of the corpus luteum. Four Steelman-Pohley bioassays for follicle stimulating hormone (FSH) were conducted utilizing 228, 23 day old prepuberal Sprague-Dawley rats to test the effect of FF on the bioassay. In the first two assays, pooled FF was tested for the presence of an FSH-binding inhibitor (FSH-BI), utilizing 108 rats. In the first assay, each rat received 20 IU of human chorionic gonadotropin (hCG), pooled with either 0, 30, 60, or 120 ug of bFSH in saline. The total amount of hormone was administered in nine .2ml sc injections given 8 hours apart and 12 rats were assigned to each dose level of bFSH. Half of the rats in each bFSH dose level also received a .3ml sc injection of bovine blood serum at each injection period, while the other half received a .3ml sc injection of the pooled luteal FF. The second assay was a replicate of the first, except that a 90ug bFSH dose level was added. All rats were killed and weighed 8 hours after the last injection. Both ovaries were removed, freed of adnexal tissues and weighed to the nearest mg. Results of the two assays were pooled. Paired ovarian weights from rats receiving 0, 30, 60, 90, and 120 ug of bFSH plus blood serum were 49, 67, 73, 78, and 104 mg, respectively, while ovaries from the rats receiving the same levels of bFSH plus FF averaged 44, 50, 64, 70, and 99 mg, respectively. Ovarian weight within the 30ug dose of bFSH was lower ($P < .02$) in rats injected with FF than in those injected with blood serum, but no significant differences were found at the other bFSH levels. When all 108 rats were considered, those injected with FF had lighter ($P < .006$) ovaries than those injected with blood serum. These results indicate that bovine FF contains a factor capable of reducing the biological action of bFSH on the ovary, possibly by inhibiting FSH binding.

In a third assay, 24 rats all received a total of 30ug bFSH plus 20IU hCG in nine .2ml injections given eight hours apart. Rats were divided into 4 equal groups and received an additional .3ml injection at each of the 9 injection periods of either: (1) charcoal extracted bovine FF, (2) non-extracted bovine FF, (3) physiological saline, or (4) bovine blood serum. Ovarian weights were similar for rats injected with charcoal extracted FF and non-extracted FF ($P < .39$) and for those injected with saline and blood serum ($P < .5$). Results of this assay indicate the factor suppressing ovarian weights was not a steroid and was not present in blood serum in concentrations high enough to have an effect.

The fourth assay involved 96 rats with half receiving 30ug bFSH plus 20IU hCG and the other half receiving 60ug bFSH plus 20IU hCG given as in the first 3 assays. Four additional treatments were administered to the 30ug FSH group: (1) .3ml bovine blood serum, (2) .15ml luteal FF, (3) .3ml luteal FF, and (4) .45ml luteal FF. The four additional treatments administered to the 60ug FSH group were: (1) .3ml bovine blood serum, (2) .3ml luteal FF, (3) .45ml luteal FF, and (4) .6ml luteal FF. All FF treatments significantly suppressed ovarian weights compared to their respective serum control. Although not significant in all cases, the higher the volume of FF administered, the more suppression obtained. Results indicate the FSH-BI protein could be competitively binding FSH receptor sites on the granulosa cells.