COMPARISON OF BOVINE FETAL AND MATERNAL GROWTH HORMONE, LUTEINIZING HORMONE AND PROLACTIN AT 90, 180 AND 260 DAYS GESTATION

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
WAYNE DWIGHT OXENDER
1971

This is to certify that the

thesis entitled
Comparison of Bovine Fetal and Maternal
Growth Hormone, Luteinizing Hormone
And Prolactin at 90, 180 and
260 Days Gestation

presented by

Wayne Dwight Oxender

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Dairy Science

Harold D. Chapman
Major professor

Date May 14, 1971

O-7639

G10034

ABSTRACT

COMPARISON OF BOVINE FETAL AND MATERNAL GROWTH HORMONE, LUTEINIZING HORMONE AND PROLACTIN AT 90, 180 AND 260 DAYS GESTATION

By

Wayne Dwight Oxender

Growth hormone (GH), luteinizing hormone (LH) and prolactin were quantified by radioimmunoassay in 37 fetal pituitaries and in umbilical arteries and veins at 90 days, 180 days and 260 days of gestation. Also, blood samples from the jugular, the uterine artery and the uterine vein of pregnant cows were taken at the same intervals.

Maternal GH increased from 5.7 ng/ml at 90 days to 10.0 ng/ml at 260 days. Serum GH averaged 2 to 5 times higher in the jugular vein sample than in the uterine vessels ($P < 0.01$). The jugular vein blood sample had more ($P \sim 0.11$) GH in cows with male fetuses (12.3 ng/ml) than in cows with female fetuses (7.3 ng/ml) at 260 days.

The fetal anterior pituitary GH increased ($P < 0.01$) during gestation (4.2, 8.9 and 18.1 µg/mg at 90, 180 and 260 days, respectively). GH for male and female fetuses

was similar at 90 and 180 days, while males averaged 12.1 and females 25.3 μg/mg at 260 days, revealed by a significant sex-age interaction ($P < 0.01$). Fetal serum GH levels increased ($P < 0.01$) with fetal age from 42, to 65 and to 103 ng/mg for 90, 180 and 260 days, respectively.

Jugular prolactin in cows averaged 220, 145 and 365 ng/ml at 90, 180 and 260 days, respectively. At 90, 180 and 260 days, cows carrying male fetuses had significantly more jugular serum prolactin than cows carrying females ($P < 0.05$).

Fetal pituitary prolactin concentration increased (72, 1150 and 2508 ng/mg for 90, 180 and 260 days, respectively, $P < 0.01$) with fetal age. Fetal pituitaries synthesized large amounts of prolactin (averaged 23.6 μg/mg/72 hr.) during incubation in vitro. Pituitaries from female fetuses synthesized three times more prolactin than males at 180 and 260 days ($P < 0.07$). Prolactin levels in the fetal serum averaged 4, 43 and 61 ng/mg for 90, 180 and 260 days of gestation, respectively ($P < 0.05$).

Maternal serum LH levels from all three blood sources were indistinguishable and averaged 0.75 to 0.89 ng/ml during gestation.

The pituitary LH concentration increased with fetal age from 323, to 474 and to 535 ng/mg for 90, 180 and 260 days respectively ($P \sim 0.06$). Pituitaries from males at 260 days contained 33% more LH than females but this difference was not significant. Fetal serum LH levels decreased

from 3.00 to 1.28 and to 0.85 ng/mg for 90, 180 and 260 days of fetal age respectively ($P < 0.01$). Unlike GH and prolactin, female serum LH averaged 3.90 ng/mg at 90 days and was significantly higher than fetal males (1.46 ng/mg) at the same age ($P < 0.01$). The female serum LH also averaged higher at 180 days but at 260 days the LH levels in the male were higher. These sex-age differences in serum LH resulted in a significant interaction of these two factors ($P < 0.01$). Fetal pituitary and serum LH concentrations were not significantly correlated ($r = -0.03$).

At birth, serum GH averaged 36 ng/ml and LH averaged 0.36 ng/ml; and neither changed significantly during the week after birth. Serum prolactin, however, averaged 101 ng/ml at birth, decreased to 42 ng/ml by the second day after birth ($P < 0.01$) and remained relatively constant to day 6 after birth.

The serum hormone gradients, maintained by the placenta between the fetus and the cow, and the fact that umbilical arterial blood serum hormone levels were indistinguishable from umbilical venous levels for GH, LH and prolactin, indicate that placental transfer of maternal GH, LH and prolactin is not a major source of fetal serum hormones. Furthermore, age and sex differences in the fetal pituitary hormones, <u>in vitro</u> synthesis of hormones and in fetal serum hormones all indicated a degree of fetal independence from maternal control. In fact, fetal influences on the maternal endocrine system were observed in this study.

COMPARISON OF BOVINE FETAL AND MATERNAL GROWTH HORMONE, LUTEINIZING HORMONE AND PROLACTIN AT 90, 180 AND 260 DAYS GESTATION

By

Wayne Dwight Oxender

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Dairy Science

1971

BIOGRAPHICAL SKETCH

of

Wayne D. Oxender

I was born in Constantine, Michigan on April 11, 1931 and received my elementary education at a small rural school. My secondary education was completed at Centreville, Michigan. I entered Michigan State College and completed one year of the agricultural shortcourse program.

I then went into partnership with my parents on a dairy farm working as partner and later as owner of a purebred Holstein herd and dairying operation. The Holstein herd was dispersed in 1961. During my farming period I gained a wife, Joan, three daughters, Barbara, Belinda and Bethany and one son, Thomas.

Desiring a veterinary medicine profession, I entered Michigan State University and received a B.S. in animal husbandry in 1966 and a D.V.M. in 1967. My experiences at Michigan State University led me to pursue postdoctoral research training. I was accepted by the Department of Dairy Science and Dr. Harold Hafs to continue my research training, shifting my major emphasis to endocrinology which I find intriguing. For the past two years I have been the

recipient of a N.I.H. postdoctoral research fellowship. During my research training I have worked part time with the Large Animal Surgery and Medicine Department. The experiences of teaching veterinary students and veterinary clinical medicine have been rewarding to me.

I am completing my Ph.D. degree with this thesis in June 1971 and anticipate the opportunity to continue as a researcher and teacher.

ACKNOWLEDGMENTS

I should like to thank the Departments of Dairy Science and Large Animal Surgery and Medicine and their chairmen, Dr. Charles Lassiter and Dr. Fayne Oberst respectively, for providing funds and facilities for my graduate studies. Also to be thanked for providing funds are my wife Joan, the National Institutes of Health General Research Support Grant FR-5623-02 and Postdoctoral Research fellowships 5-FO2-HD42436-01 and 5-HD42436-02. But to my friend and advisor, Dr. Harold Hafs must be extended a debt of gratitude for providing me support, encouragement and enthusiasm to complete my graduate studies. Also I am grateful for the advice and support of my committee members, Drs. R. L. Anderson, C. E. Meadows and C. K. Whitehair.

This thesis, as the reader will realize, is not the output of one person, but involved the participation of many individuals. They include radioimmunoassay antisera for LH, GH and Prolactin provided by Dr. Lloyd Swanson, Dr. Roger Purchas and Dr. H. Allen Tucker, respectively. The purified hormones for radioiodination and standards were supplied by Dr. Leo Reichert and the N.I.H. Endocrinology Study Section. Providing assistance for R.I.A. methods were James Koprowski and Dr. Lee Edgerton. Dr.

Ec
t
Le
Sw
Ch
ar
cu

Ba
la
ef
th

Edward Convey assisted with the anterior pituitary incubations and assay techniques. Several people including Dr. Lee Edgerton, Winston Ingalls, James Koprowski, Dr. Lloyd Swanson and Joseph Zolman helped collect samples. Drs. Chris Miller and David Morrow assisted with the surgeries and pregnancy examinations. Dennis Armstrong aided in procuring and transporting the animals.

Finally, I would thank my wife Joan and children Barbara, Belinda, Bethany and Thomas for assistance in the laboratory but more importantly for accepting my transient efforts as a husband and father during the completion of this thesis project.

TABLE OF CONTENTS

LIST OF TABLES

Table | Page

LIST OF FIGURES

Figure Page

LIST OF APPENDICES

INTRODUCTION

What is the degree of fetal autonomy during development within the maternal environment? One problem encountered by endocrinologists and reproductive physiologists studying reproduction and developmental biology is whether the hypothalamo-hypophyseal system of the fetus participates in the maturation of the genital apparatus of the fetus. For instance, to what extent does the fetal hypothalamo-hypophyseal system and its secretions control somatic growth, the development of the gonads and the differentiation of the genital duct system? There is limited knowledge of the hormone levels of the fetal endocrine glands and the ability of the glands to synthesize and release hormones in utero. As a consequence, whether the prenatal endocrine secretions are identical to their adult counterparts or if the fetal responses to endocrine stimuli are similar to the response elicited postnatally are undetermined.

Male or female development appears to be gradual transition. Genetic sex of the individual is determined at conception, but gonads, genital ducts, accessory gland development and hypothalamic function can each be recognized at sexually indifferent stages. In other words, except for

the genetic sex of an individual, endocrine secretions apparently determine at least in part male or female differentiation of the gonads, genital ducts, accessory glands and hypothalamus. Parts of sexual differentiation appear to be reversible while others, once differentiated, appear to be permanent.

Major advances have been made recently in the area of endocrinology allowing determination of levels of hormone in fetal and maternal blood. Fetal pituitary hormone content and ability to synthesize hormones also can be quantified by recently developed radioimmunoassays (RIA). This has been a major breakthrough in endocrinology, including developmental biology. Whereas formerly, researchers quantified hormones crudely into "gonadotrophic activity," it is now possible to identify and quantify specific hormones in most endocrine organs and biological fluids.

With the above questions about sexual development and with the new techniques for quantifying hormones, this project was developed, initiated and completed. The bovine fetus was chosen as the subject of this project because of availability and convenient size for study. Endocrine developments of the bovine from the birth through puberty have been the subject of comprehensive studies in this laboratory in the past. This fetal developmental study was designed to extend our knowledge of the endocrine and reproductive changes of the bovine to the period before

birth. The object was to study at least five male and five female fetuses, and their dams, near the end of each trimester of pregnancy.

While this study may not provide final answers, knowledge gained from these studies may find practical application in a number of current endocrine malfunction problems in cattle. Furthermore, some human endocrine malfunctions lead to similar abnormal fetal and neonatal development.

Increasing reproductive efficiency of animals would increase the supply of animal protein for food, with impact on malnutrition due to the lack of dietary protein.

REVIEW OF LITERATURE

A. Historical

> The fact is that animals, if they be subjected to a modification in minute organs, are liable to immense modifications in their general configurations. This phenomenon may be observed in the case of gelded animals: only a minute organ of the animal is mutilated, and the creature passes from the male to the female form. We may infer, then that if in the primary conformation of the embryo an infinitesimally minute but absolutely essential organ sustains a change of magnitude one way or the other, the animal will in one case turn to male and in the other to female: and also that if the said organ be obliterated altogether, the animal will be neither one sex nor the other.

This quotation from Aristotle from over 2000 years ago (Smith and Ross, 1910) has to be one of the earliest published observations of endocrine function. Evolution of knowledge covering development of animal intersexes prior to 1779 was published in a treatise by Hunter and has been reviewed by Short (1969).

McClung (1902) was the first to describe the genetic basis for sexuality. He found that all but one pair of chromosomes were identical in male and female cells. Further comparison of this non-identical pair of chromosomes revealed that chromosomes from male cells had one smaller chromosome in the pair of sex chromosomes, while the two chromosomes were identical from female cells. Later, Morgan and Bridges demonstrated that the autosomes also can influence sexual development in Drosophila (Bridges, 1939).

Fetal development studies received early assistance from Bouin and Ancel (1903) when they reported that the male fetal testis was a secretory organ. McCord (1915) was one of the first to demonstrate oxytocic activity in fetal pituitaries and epinephrine activity in fetal adrenal glands. The interesting natural phenomenon of the sterile bovine freemartin, a female born twin to a male, was hypothesized to be the result of hormone exposure during prenatal development by the now classic studies of Lillie (1916) and Keller and Tandler (1916). Comparative endocrinological studies of fetal development using insulin from the bovine fetal pancreas to depress blood glucose in a pancreatectomized dog have been reported by Banting and Best (1922) and by Hogben and Crew (1923). The latter authors investigated the cause of the pathological "bulldog" fetus in Dexter cattle. The fetal thyroid gland from "bulldog" fetuses contains the same thyroid activity as normal calves when determined by metamorphic changes in axolotls (a small amphibian).

B. Fetal Surgery

As early as 1803 surgical procedures were developed to observe antenatal movements establishing the feasibility of surgery as a research technique (Swenson, 1925).

To study the contribution of the fetal pituitaries to fetal development, Jost (1947) and Wells (1947) used fetal decapitation. Newer techniques for fetal hypophysectomy

by using radioactive pellets (Hutchinson et al., 1962), electrocoagulation (Liggins et al., 1967) and surgical excision (Kraner and Parshall, 1969) are more specific, but remove all pituitary hormones simultaneously.

A technique of marsupializing the fetus for complicated surgical techniques has been reported by Jackson and Egdahl (1960). Fetal surgical techniques for skin grafting, splenectomy, gonadectomy and thymectomy have been described by Kraner and Parshall (1969). Several surgical approaches for fetal immunological studies in primates have been successful (Parshall and Silverstein, 1969). Evidence that fetal endocrine activity may have far reaching consequences is abundant. For example, fetal adrenalectomy causes prolonged gestation in ewes (Drost and Holm, 1968).

Another very successful surgical technique for studies of fetal endocrine development is catheterization of the fetal vascular system (Kraner and Parshall, 1969; Snow and Tyner, 1969). Chronic catheterization has been used to study fetal plasma corticosteroids (Bassett and Thorburn, 1969), insulin induced hypoglycemia in the ovine fetus and placental transfer of insulin (Colwill et al., 1970), placental transfer of GH (Gitlin et al., 1965), and placental transfer of LH and fetal response to luteinizing hormone releasing factor (Foster, 1971). Placental transfer of drugs using the above techniques recently has been

repor

nilam

durin

vivo

and M

develo

of ra

amnio

ated h

blood

can be

ment.

nancy

tem.

separa

conclu

on the

et al.

cow ha

non-pr

oping

reported by Almond et al. (1970); fetal levels of sulfanilamide were maintained at 60% of the maternal levels during constant infusion of the mother.

Fetal metabolic studies and placental transfer in vivo using radioactive compounds were reported by Diczfalusy and Mancuso (1969). An artificial fetal environment was developed by Zapol et al. (1969) to study fetal metabolism of radioactive compounds. A synthetic fluid similar to amnionic fluids suspends the fetus in vitro, while oxygenated blood is perfused through the fetus via umbilical blood vessels.

As indicated above, numerous surgical procedures can be used as aids to the study of fetal endocrine development.

C. Maternal Hormones during Gestation

Zelenik (1965) reviewed the endocrinology of pregnancy which must be viewed as a completely integrated system. Evaluating one hormone alone or one hormone system separately probably will continue to give isolated and inconclusive information.

The early influence of the developing bovine embryo on the maternal endocrine system was reported by Shemesh et al. (1968). On day 19 after conception, the pregnant cow has progesterone levels three-fold greater than the non-pregnant cow (Shemesh et al., 1968). Thus, the developing fetus affects the maternal endocrine system.

Furthermore, the extent of this affect appears to be related to the sex of the fetus; women with female fetuses have human chorionic gonadotropin (HCG) serum levels more than two-fold greater than women with male fetuses after 190 days of gestation (Brody and Carlström, 1965). Urine pregnanediol excretion is greater in women with male fetuses suggesting that sex differences of the fetal placenta may be responsible for differential metabolism of steroids (Rawlings and Krieger, 1964). Resko (1970) found higher plasma testosterone concentrations in primates with male fetuses than in those with female fetuses.

Fetal sex influences on the maternal endocrine system are not the only fetal characteristics that have been reported. MacMillan (1970) found significantly reduced human chorionic somatomammotropin in the plasma and placenta of mothers that delivered abnormally small babies.

1. Pituitary Hormones

Maternal pituitary hormones are essential to maintain pregnancy in most species during the first half of gestation. In early pregnancy, progesterone secretion is controlled by pituitary hormones, but placental hormones apparently replace this pituitary function later (Jaffe et al., 1969). Pregnancy proceeds normally in females that are hypophysectomized near mid-gestation in several species (Hutchinson et al., 1962). Chez et al. (1970) hypophysectomized primates in the second trimester of gestation without causing abortions.

in wo

chang

peake

dent

the f

lacti

(Schar

48 hou

2. St

adrena

must a

and es

vary c

interr

and pr

abort

after

viewed

gestero

to term

pregnanc

as gesta

Faiman et al. (1968) found no change in serum FSH in women during gestation. Pituitary FSH levels did not change in the sow during gestation, and while LH levels peaked on day 18, proportionately little change was evident during gestation (Melampy et al., 1966).

Bovine serum LH averaged less than 1 ng/ml during the first 60 days of gestation (Schams, 1969). Serum prolactin increased sharply to 880 ng/ml before parturition (Schams and Karg, 1970), but this peak disappeared within 48 hours.

2. Steroid Hormones

In the nonpregnant female, only the ovaries and adrenal glands produce steroid hormones while the placenta must also be considered during pregnancy. Progesterone and estrogen production by the ovaries during pregnancy vary considerably among species. Ovariectomy does not interrupt gestation in the guinea pig, cat, sheep, horse, and primates while the mouse, rabbit, goat and sow always abort following ovariectomy. The cow can be ovariectomized after 200 days of gestation without causing abortion (reviewed by Amoroso and Finn, 1962).

Stabenfeldt et al. (1969) found bovine plasma progesterone levels increased nearly 50% from mid-gestation to term. Urinary estrogen elimination increases during the pregnancy period in humans and serum levels also increase as gestation proceeds (Diczfalusy and Mancuso, 1969;

Younglai and Solomon, 1969). Plasma corticosteroids do not change in the ewe during gestation (Bassett and Thorburn, 1969).

D. Placental Function during Gestation

The placental permeability properties are unique. They can prevent transfer, selectively or actively transfer, or be completely permeable to a given biological compound. Considerable metabolic and synthetic activity is also associated with the placenta. In spite of the important placental functions, this specialized tissue is only a temporary organ. Its genetical derivation is from two sources. The endometrium of the mare had gonadotophic activity when the embryo was only 1.8 cm long (Catchpole and Lyons, 1934), and gonadotrophic activity was found in the incubation media after human placenta had been incubated *in vitro* (Gey *et al.*, 1938).

1. Hormone Synthesis

Human chorionic gonadotropin (HCG) is the placental hormone that has been most widely studied. Aschheim and Zondek (1927) showed that it had gonadotrophic activity and since that time many studies have contributed to our present knowledge about HCG (reviewed by Brody, 1969). In addition to HCG, another protein hormone human chorionic somatomammotropin is synthesized by the human placenta. This hormone is sometimes called human placental lactogen

(Grur
MacM
hormo

bovir
1967;
cate
teror
and b
geste

centa
the n
(Pier
place
gesti
stero
scurc

2. T

prope
among
permea
determ
mother
gonado

(Grumbach et al., 1968; Gitlin and Biasucci, 1969; MacMillan, 1970). Human placental lactogen has both growth hormone and prolactin activity.

Progesterone is synthesized in vitro by sheep, bovine and human placental tissues (Ainsworth and Ryan, 1967; Younglai and Solomon, 1969). In vitro studies indicate that human placental preparations metabolize progesterone producing several derivatives. In contrast sheep and bovine placental preparations possess very little progesterone metabolic activity (Ainsworth and Ryan, 1967).

Estrogens are synthesized by bovine and goat placental preparations in vitro indicating the presence of the necessary enzymes for aromatization of steroids (Pierrepoint et al.,1969a; Ainsworth and Ryan, 1970). The placenta in most species, however, is a source of progestins, estrogens and androgens. Precursors for these steroid hormones may come from either fetal or maternal sources.

2. Transfer of Hormones

The type of placenta varies and the permeability properties and hormone metabolic activity appear to differ among many species. Since the placenta is selectively permeable to certain hormones it has been difficult to determine whether the hormone is from the fetus or the mother. Goodman and Wislocki (1933) could not detect any gonadotrophic activity in fetal fluids after pregnant cats

and

ext

int

of

He

mat

and

(RI

wit

pit

pla

cro

tra

the

et

lin

the

als

hor

hor

197

cur

mot

pla

syn

late

and rabbits were injected with HCG or anterior pituitary extract. The technique for injecting ^{131}I-Growth hormone into the umbilical cord and determining the distribution of the radioactivity was developed by Gitlin et al. (1965). He showed that no radioactivity could be found in the maternal circulation. Gonadotropins in umbilical cord and maternal blood serum were measured by radioimmunoassay (RIA) (Faiman et al., 1968). Both of these studies agreed with the earlier conclusions of Mitskevich (1962) that pituitary hormones do not appear to be transferred by the placenta. Foster (1971) demonstrated that LH does not cross the placenta in either direction. However, specific transfer of proteins must occur because insulin crosses the placental barrier readily (Mitskevich, 1962; Gitlin et al., 1965; MacMillan, 1970) and maternal gamma globulins are transferred to the fetus but albumin cannot cross the placenta (Gitlin et al., 1965). Thyroid hormone is also transferred by the placenta but thyroid stimulating hormone (Mitskevich, 1962) is not.

Extensive studies using doubly labeled steroid hormones (Diczfalusy and Mancuso, 1969; Pasqualini et al., 1970) showed that most steroid hormones and their precursors appear to be transferred by the placenta from mother to fetus and from the fetus to the mother. The placenta appears to be the source of increasing estrogens synthesized from precursors supplied by the fetus during late gestation in humans.

treat

that

(1969

sulti

gen i

the c

1969).

steroi

born t

nancy

Jost a

steroi

in the

used d

reprod

tion ra

fed the

et al.,

stage o

conside

females

volves t

raternal

Fetal abnormalities produced by steroid hormone treatments of the mother during gestation also indicate that steroids are transferred to the fetus. Shane et al. (1969) fed pregnant dogs testosterone and all of the resulting female offspring were pseudohermaphrodites. Estrogen injections in pregnant beagles caused malformation of the coxofemoral joints in the pups (Gustafsson and Beling, 1969). Oral contraceptive pills contain a variety of steroids and have caused virilization of female children born to women who have taken contraceptives during pregnancy (reviewed by Jones and Wilkins, 1960; Ferreira, 1969). Jost and Moreau-Stinnakre (1970) fed two contraceptive steroids to pregnant rats and produced genital anomalies in the female offspring. However, not all steroid hormones used during pregnancy prove to be detrimental to the future reproductive performance of the offspring. Normal conception rates were recorded for heifers from cows that were fed the synthetic progestin, melengestrol acetate (Schul et al., 1970).

The species, kind of steroid, level of steroid and stage of fetal development are all factors that must be considered when steroids are to be administered to pregnant females.

E. Parturition

The final event for the developing fetus which involves the intimate interactions of the fetoplacental-maternal systems is parturition. Many workers have searched

for the factors controlling this event and several factors appear to be involved. Only a few of the more recent reports on the fetal contribution to the initiation of parturition are listed here. Liggins et al.(1967) produced prolonged gestation in sheep by destruction of the fetal pituitary and reported that developmental failures of the fetal pituitary caused by ingestion of Veratum californicum in pregnant ewes also delayed parturition. Fetal adenohypophysealaplasia appeared to be caused by a genetic defect in Guernsey cattle and results in prolonged gestation. In addition to adenohypophysealaplasia, the fetal thyroid and adrenal cortex were hypoplastic in cattle (Kennedy et al., 1957) and in humans (Benirschke, 1956). Fetal adrenalectomy also caused longer gestation periods in sheep (Drost and Holm,1968).

Fetal lamb corticosteroid levels determined by umbilical catheterization revealed a six-fold increase in fetal corticosteroids from 130 to 150 days gestation (Bassett and Thorburn, 1969). Adams and Wagner (1970) reported plasma corticoid levels in the pregnant cow increased 100% the last four days of gestation. While the fetal adrenal apparently participates in initiation of parturition, how the fetal adrenal controls maternal uterine muscle remains to be determined.

F. Fetal Sexual Development

During the fetus's intra-uterine development, the endocrine glands and the hormonal receptor organs

diffe

for p

show u

and s

Early

(1903)

to the

activi

Jost (

tary h

partic

and Pi

uterine

they pl

fetus?

ing rec

transpla

et al. (

an inhib

entiatio

supporte

acquires

sex.

differentiate, and at birth they are more or less ready for postnatal functions. Before birth, some fetal glands show unquestionable cytological signs of secretory activity and some organs seem to have been stimulated by hormones. Early observations and interpretations by Bouin and Ancel (1903), Lillie (1916), and Keller and Tandler (1916) led to the belief that the fetal testes produced androgenic activity. Subsequent studies by Wells (1946, 1947) and by Jost (1947) using fetal decapitation and exogenous pituitary hormones concluded that the fetal pituitary and testes participate in sexual differentiation (reviewed by Jost and Picon, 1970).

Do the fetal glands only prepare, during intrauterine life, for their later postnatal functions or do they play an indispensible part in the development of the fetus? Do maternal hormones exert effects on the developing receptor organs?

In experiments involving fetal castrations, organ transplants and exogenous androgens, Jost (1955) and Neumann et al. (1969) concluded that the fetal testes have both an inhibitory and a stimulatory action on sexual differentiation. Additional research reviewed by Jost (1965) supported the above conclusions. The castrated fetus acquires feminine sexual features irrespective of genetic sex.

Three aspects of fetal development that appear to be controlled by fetal pituitary trophic hormones are sexual differentiation, thyroid colloid synthesis and liver glycogenesis (Jost, 1966; Jost and Picon, 1970).

1. Gonads

Gonad differentiation begins with formation of primordial germ cells in the yolk sac lateral to the base of the viteline artery with subsequent movement of these primordial germ cells into the embryo proper. The first distinct germinal epithelium is found in 28-day bovine embryos. Migration and elongation by mitotic division of the germ cells forms the genital ridge by day 30 in the bovine. Continued rapid expansion of the genital ridge results in a ballooning of the developing gonad. At about 40 days (Krehbiel, 1963; Erickson, 1966; Gier and Marion, 1969; Matschke and Erickson, 1969) the sexually indifferent stage of gonad development ends and sex differentiation begins. By 42 days of bovine pregnancy, ovarian cortex is formed in the female or seminiferous cords in the male gonad and Leydig cells appear at 44 days (Gier and Marion, 1969). Macroscopic sex determination is possible at day 46 in the bovine embryo (Krehbiel, 1963). Macnaughton (1969) reported that the fetal rabbit gonad is differentiated at 15 days. The gonad differentiation appears to take place very early in fetal development and may be primarily controlled by genetic factors.

Bascom (1923) investigated fetal calf and pig testes and hypothesized that the interstitial cells were active *in utero*. Bovine fetal testicular extracts were a more potent source of androgens than adult testicular extracts (Koch, 1931). Ovarian and testicular extracts contained estrogenic activity but lacked androgenic activity (Cole *et al.*, 1933). Wells (1946) used exogenous gonadotropin to stimulate an increase of Leydig cells in the fetal rat testis.

Incubation of bovine ovaries with labeled steroid precursors (Roberts and Warren, 1964) indicated that fetal ovarian tissue is capable of some steroid transformations similar to the adult bovine ovary (Sweat *et al.*, 1960). However, there is no evidence that fetal ovarian secretions are essential for sexual differentiation.

In contrast fetal testicular secretion appears to be essential for normal male sexual differentiation. Benirschke and Bloch (1960) failed to demonstrate testosterone in testes in bovine fetuses that were around 110 days old. In contrast, Struck and Karg (1967) measured both testosterone and androstenedoine in fetal calves after 120 days of gestation. Testosterone levels of both fetal lamb (Attal, 1969) and calf testes (Struck and Karg, 1967) appear to peak in the second trimester of gestation and then decrease as gestation advances. Umbilical cord blood levels of testosterone are higher in males than in females in fetal rhesus monkeys (Resko, 1970).

The observation of periodic endocrine activity of fetal glands during development was made by Jost (1947) originally and was supported by several other studies (Van Wagenen and Simpson, 1954). Fetal testicular secretion of androgens appears to be well documented, but their action and especially the integration of androgens with the fetal endocrine system remains obscure.

2. Genital Tract

Normally, the tubular and external genitalia of both sexes develop to an indifferent stage and then differentiate. However, all degrees of intersexual development are possible between the normal male and female. In males, the Wolffian or mesonephric ducts develop into the epididymis, vas deferens and seminal vesicles. The prostate gland and bulbourethral gland originate from the urogenital sinus. The Mullerian duct regresses in the male. In contrast, the female infundibulum, oviduct, uterus and vagina all develop from the Mullerian ducts and the Wolffian ducts regress. Male or female external genitalia differentiates from a common origin.

Much research interest has centered on genital duct differentiation and its exogenous control. Possibly, the existence of the bovine freemartin (see Freemartin development) has had a stimulatory effect on research in this area.

Price and Pannabecker (1959), in a unique experiment, used embryonal genital tracts cultured *in vitro* to discover that testosterone would prevent female organogenesis. Androgens and anti-androgens have been used by Neumann *et al.* (1969) to induce intersexuality.

Androgens or testicular secretions appear to be necessary for normal male genitalia development. The need for more precise information on hormone caused abnormalities is evident.

3. Hypothalamus

If one assumes that the male and female pituitary are similar and that only the anterior pituitary of the female releases gonadotrophic hormones in a cyclic pattern while the male gonadotrophic release pattern is tonic, then higher brain centers must control pituitary gonadotrophic release (Harris and Jacobsohn, 1951). The hypothalamus apparently is responsible for female cyclicity and indirectly for estrus behavior (reviewed by Young, 1961 and 1966; Levine, 1971).

Sexual differentiation of the hypothalamus appears to involve irreversible alteration of sensitivity to steroid hormones (Barraclough, 1967). The age at differentiation varies with species. The age in the hamster is 1-3 days postnatally (Gorski, 1968; Alleva *et al.*, 1969), but in the guinea pig it occurs prenatally (Young, 1961). Differentiation of the hypothalamus appears to be prenatal in

species such as pigs and calves that have more mature fetuses at birth (Zimbleman, 1964).

Androgens seem to be necessary to prevent the development of the female cyclic pattern of gonadotrophic secretion. Females exposed to androgens during the critical age-period are said to be androgen sterilized. Androgen sterilized females do not show evidence of cyclic activity as judged by vaginal smears. Females that have been so sterilized fail to mate, ovulate or conceive and may show male behavioral aggressiveness (Harris and Levine 1965). Dorfman (1967) could prevent the androgen sterilization of female rats by simultaneous administration of progesterone. Estrogen exposure to male fetuses or neonates causes testicular atrophy presumably through interruption of gonadotrophic stimulatory mechanisms possibly at the hypothalamic level. This action of estrogen is prevented by progesterone (Dorfman, 1967).

Eguchi and Morikawa (1968) approached the fetal gonadotropin secretion problem by the use of parabiotic twin studies. If they used intact female fetuses and decapitated males they observed testicular atrophy suggesting that the male fetal pituitary releases gonadotropins.

Large doses of an oral contraceptive (Lynestrenol and Mestranol) to lactating hamsters caused permanent sterility in the nursing offspring (Czyba et al., 1969). These studies suggested that the estrogenic activity of

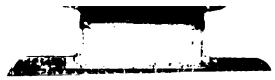
the contraceptive agent may have acted directly on the neonatal gonads to cause sterility in the case of the nursing hamsters.

Hypothalamic control of pituitary function has been studied by controlled deafferentiation of hypothamic areas with the Halasz knife (Halasz and Gorski, 1967). Deafferentiation studies have shown that cyclic LH release in females is controlled by brain centers other than the median basal hypothalamus (Halasz and Gorski, 1967).

The sexual differentiation of the hypothalamus by exposure to steroid hormones at critical periods seems well established in rats and in a few other species. In addition, marked behavioral patterns of humans appear to be influenced by hormonal stimuli (reviewed by Levine, 1971).

4. Mammary Gland

Mammary gland development is also sexually dependent; however there are some stages in development which appear to be reversible. Cupceancu et al. (1969) reported that administration of several synthetic progestogens to pregnant rats prevented normal mammary gland development in both female and male fetuses. Similar results were obtained by Jean (1969) and by Jean and Delost (1969a) using androgens and estrogens on pregnant mice. Prolactin did not cause fetal mammary gland abnormalities when given to pregnant mice (Jean and Delost, 1969b).



5. The Freemartin

The freemartin appears to be a unique abnormality in sexual differentiation of the bovine and has been the subject of numerous investigations. Early scientists attempted to determine the cause and more recent efforts have failed experimentally to produce a freemartin. Hunter in 1779 reported on intersexes in several species but it was not until 1916 that a fetal hormone was theorized to be the cause of freemartinism. Lillie (1916) and Tandler and Keller (1916) independently arrived at the conclusion that the cause was hormonal transfer to the female fetus from the male fetus. A classic paper by Lillie (1917) compared his findings with all the previously advanced theories. A popular theory at that time was that the sterile female was a genetic male or monozygotic twin. Lillie's conclusions were: (1) the twins were of separate zygotic origin, (2) vascular anastomosis of placental vessels was present, (3) the sterile female was limited to the bovine because of the type of placentation, and (4) the fetal testis developed earlier than the ovary. Injections of dyes showed a common placental circulation between the male and female fetuses of the bovine (Lillie, 1917), in sharp contrast to the separate circulatory systems observed in sheep fetuses even when twin fetuses share a cotyledon (Mellor, 1969). Chaplin (1971) did an extensive histological study of freemartins and substantiated the

wide variability observed in freemartin gonads and genitalia development. According to Willier (1921) the degree of transformation of the freemartin ovary into a testis histologically was related to the degree of masculinization of the external genitalia.

Studies of the metabolic activity of freemartin gonads *in vitro* were reported by Hoffman and Martin (1968) and by Pierrepoint *et al*. (1969b). The former authors reported synthesis of androstenedione from progesterone while the latter authors found that the rate of metabolism of dehydroepiandrosterone was intermediate to that of the normal ovary and testes.

Administration of several androgens at varying levels and periods of gestation all produced similar results (Mason *et al*., 1958; Hurst, 1962; Jost *et al*., 1963; Jainudeen and Hafez, 1965). All of the above researchers observed some masculinization of external genitalia of the developing female fetus. However, in contrast to the freemartin, the ovaries were always normal.

Chimerism of germ cells in freemartin gonads has been reported by Ohno (1969). Fechheimer *et al*. (1963) and Stewart (1965) hypothesized that this germ cell mosaicism in the gonad caused the freemartin anatomical defects by local action on the germinal ridge. Ohno (1969) favored the hormonal theory as a cause of freemartin development.

It is possible that hormonal influences from the male twin testes and germ cell chimerism with its effect on the germinal ridge each contribute to the development of a freemartin. The etiology is still unknown.

G. Fetal Pituitary Hormones

Male rat pituitaries when transplanted into females release gonadotropins in a cyclic manner according to Harris and Jacobsohn (1951). The evidence suggests that the pituitary does not participate in sexual differentiation but that sexual differences in secretion and content of pituitary hormones are controlled by factors outside the pituitary. Hypothalamic releasing factors, inhibitory factors, pituitary hormones and gonadal hormone feedback mechanisms are all controls on the pituitary function.

Early research on bovine fetal pituitaries by McCord (1915) indicated the presence of oxytocic activity in 56-day fetuses. McCord suggested that the fetus was under the influence of its own glands *in utero*. Smith and Dortzbach (1929) used pituitary extracts from 17-cm fetal pigs to cause precocious sexual development in immature mice and extracts from 9-cm pig fetuses to stimulate growth in hypophysectomized rats. He concluded that GH and gonadotropins were separate hormones and both were present in the fetal pituitary. Gonadotrophic activity of fetal pituitary extracts have been recorded for the horse (Hellbaum, 1935) and for the cow (Bates *et al.*, 1935).

Bates also found prolactin and thyroid stimulating activity in the bovine fetal pituitary, indicating an autogenous hormone source for the fetus.

Another accident of nature is the human anencephalic fetus. These fetuses may develop without a brain, hypothalamus and/or pituitary. Hypoplasia of the fetal adrenals and testes are common pathological changes associated with anencephalic humans and indicate the lack of trophic hormone stimulation (Benirschke, 1956; Blizzard and Alberts, 1956; Brewer, 1957; Zondek and Zondek, 1965). Liggins and Kennedy (1968) noted hypoplasia of the adrenals, testes and thyroid when the sheep fetal pituitary was removed. A reciprocal functional relationship between the hypophysis and the adrenals during fetal development was demonstrated by Kitchell and Wells (1952). Electron microscopic studies by Hatakeyama (1969) confirmed activity of the hypothalamic-pituitary-adrenal axis in the human fetus by mid-gestation. Mitskevich (1962) also concluded that the fetal pituitary-thyroid function is established during gestation in the rabbit.

Bioassays demonstrated GH, LH, FSH, TSH, MSH, ADH, prolactin and oxytocin activity in the fetal pituitary of various species (reviewed by Levina, 1968 and Macnaughton, 1969). Fetal pituitary FSH was detected in the rat (Corbin and Daniels, 1967), in sheep (Mauleon and Reviers, 1969), and in man (Levina, 1968). Fetal serum FSH levels were

reported by Faiman et al. (1968) for umbilical cord blood samples but whether the fetal serum FSH originated in the dam was not determined.

Fetal pituitary LH concentrations were reported for man (Levina, 1968), for sheep (Mauleon and Reviers, 1969; Foster, 1971), and for cattle (Karg, 1967a, 1967b). Mauleon and Reviers (1969) found pituitary LH content in the male similar to that in the female fetus using bioassay, however Foster (1971) found that LH levels of males were higher than those of females using RIA. The LH-FSH ratio in fetal pituitaries differs in males and females for humans (Levina, 1968) and for sheep (Mauleon and Reviers, 1969). When human fetal pituitaries were transplanted into adult hypophysectomized rats (Levina, 1968), gonadotropin secretion of the fetal human pituitaries stimulated the rat gonads.

Serum levels of LH in fetal lambs increased after injection of porcine hypothalamic extract (Foster, 1971) indicating the fetal lamb pituitary is capable of responding to releasing factors.

RIA techniques will allow more precise research on individual pituitary hormones and their release patterns in the fetus. Each fetal hormone should be compared to the related endocrine environment to more accurately understand fetal pituitary function.

1. Pituitary Tissue Culture

Pituitary synthesis and secretion can be studied in tissue cultures. Gey et al. (1938) incubated fetal pituitary tissues and tested the incubation medium for gonadotrophic activity. He could not detect activity in a bioassay. However, he reported that microscopic examination indicated slight gonadotrophic activity. Better methods of pituitary explant culture have since been developed (Meites et al., 1961; Piascek and Meites, 1967). Meites et al. (1961) reported rapid synthesis and release of prolactin by rat pituitary explants. Radioactively labeled amino acids were incorporated into GH, FSH, LH, and TSH during incubation of human fetal pituitary tissues (Gitlin and Biasucci, 1969). In their study, GH was the earlist pituitary hormone shown to be synthesized by human fetal pituitary cultures. Labeled GH was synthesized in cultures of 60-day fetal pituitaries. Gailani et al. (1970) detected GH, LH and TSH in human fetal pituitary culture media using RIA. Growth hormone measured in the media by RIA also was capable of producing growth of the rat tibia, thereby showing a correlation of the immunological and biological assays.

Pituitary tissue culture techniques have been important methods to test for releasing factors and steroid hormone feed-back controls on the anterior pituitary. Caution must be used in interpreting in vitro results with the in vivo pituitary function.

2. Luteinizing Hormone (LH)

Gonadotrophic activity in fetal pituitary extracts from the pig (Smith and Dortzbach, 1929; Melampy et al., 1966), the horse (Hellbaum, 1935), the sheep (Mauleon and Reviers, 1969; Foster, 1971), the cow (Bates et al., 1935; Karg, 1967a, 1967b) and in man (Levina, 1968; Rice et al., 1968) have been reported. Gitlin and Biasucci (1969) used immunoelectrophoretic techniques to identify in vitro synthesis of labeled LH by human fetal pituitary explants which had been incubated with radioactive labeled amino acids. Immunofluorescent techniques were used by Dubois and Mauleon (1969) to identify LH in anterior pituitary cells of a 49-day lamb fetus. Foster (1971) used RIA to quantify LH in fetal sheep pituitary homogenates and found that pituitary LH increased faster in males than in females. This increase in fetal pituitary LH concentration with increased fetal age agrees with the bioassay data for sheep (Mauleon and Reviers, 1969) and also for the cow (Karg, 1967a, 1967b).

In early studies researchers were unable to detect gonadotropic activity in fetal blood and serum using bioassays. Foster (1971) used RIA to measure the serum LH levels in fetal lambs from 55 days through parturition and detected increased LH up to 100 days and then a decrease as parturition approached. I found no reports of fetal bovine serum LH in the literature.

Sexual differences in fetal pituitary LH concentration have been observed in the cow (Karg, 1967b) and in the

sheep (Foster, 1971). Mauleon and Reviers (1969) found a sexual dimorphism in LH:FSH ratios in fetal lamb pituitaries similar to that observed by Levina (1968) in human fetal pituitaries. RIA and catherization of developing fetuses makes possible a more accurate determination of LH during fetal development.

3. Growth Hormone (GH)

In a review, Jost (1966) concluded that GH is not required for rabbit fetal growth, but is required for synthesis of glycogen by the fetal liver. These conclusions were derived mainly from studies on the changes following decapitation of the rabbit fetus. Heggestad and Wells (1965) studied prenatal growth of decapitated rat fetuses and concluded that the rat fetus requires GH for normal growth. However, exogenous GH would not cause added growth in normal fetuses. The fetal monkey appears to grow at a nearly normal rate after hypophysectomy (Chez et al., 1970). Decapitation of sheep fetuses appeared to retard bone growth, although most of the other parts of decapitated fetuses developed normally (Liggins et al., 1967; Liggins and Kennedy, 1968). Nanagas (1925) studied the body growth patterns of human anencephalic and normal fetuses and discovered disproportionate growth of the anencephalic fetuses.

Smith and Dortzbach (1929) found that anterior pituitary extracts from 9- to 11-cm pig fetuses stimulated

growth in hypothysectomized rats. Biologically active GH also was detected in human fetal pituitaries by the rat tibia test (Rice et al., 1968). Pavlova et al. (1968) used a hemagglutination inhibition test to quantify GH in human fetal pituitary homogenates and correlated these results with histological studies of the anterior pituitary. They found trace amounts of GH at 60 days of gestation and increasing quantities up to 140 days, paralleling an increase in pituitary acidophils. Immunofluorescent staining of fetal lamb anterior pituitary tissues identified GH in the tissues from 58-day or older fetuses (Stokes and Boda, 1968); differential staining of pituitary tissue revealed two types of acidophils, one which produced prolactin and the other GH.

Immunofluorescent techniques also were used to study bovine fetal pituitaries (Meneghelli and Scapinelli, 1962). They found that 100-day fetuses had GH-containing acidophils which increased in number as the age of the fetus increased.

Another method of studying fetal GH is in vitro incubation of pituitaries. Brauman et al. (1964) cultured human fetal pituitaries for five weeks and found that GH production decreased as the incubation time increased. Radioactive amino acids were incorporated into human growth hormone by 60-day fetuses using in vitro pituitary tissue cultures (Gitlin and Biasucci, 1969). Biological and

immunological human GH activity was present in the culture media of fetal anterior pituitary cell cultures for periods of incubation as long as 150 days (Gailani et al., 1970).

The plasma GH levels of fetal lambs were 10-fold higher than in the mother and increased from 40 ng/ml at 110 days to 120 ng/ml at 140 days of gestation (Bassett et al., 1970). Hypophysectomy of fetal lambs resulted in disappearance of plasma GH, and exogenous GH given to fetal lambs was degraded at a rate similar to that of endogenous GH (Bassett et al., 1970). I conclude that fetal plasma GH is higher than maternal plasma GH, and although fetal GH has similar biological activity to that in adults, its function in fetal development has not been established.

4. Prolactin

In 1935, Bates et al. reported that the bovine fetal pituitary contained two to three times more prolactin activity than older cattle. In contrast, Reece and Turner (1937) reported bovine fetal pituitaries contained less prolactin activity than older calves. Lyons (1937) measured prolactin activity in urine from new born human babies and put forth the premise that "witches milk" or mammary gland secretions by many newborn babies is caused by high prolactin levels in the fetus (reviewed by Smith, 1959). Smith (1959) also stated that premature babies seldom have mammary gland secretions at birth.

Immunofluorescent location of the prolactin-containing acidophils in fetal sheep pituitaries could not be observed until 80 or more days of gestation; they were always less prevalent than GH acidophils (Stokes and Boda, 1968).

Human fetal pituitaries produce more prolactin than GH *in vitro*. GH and prolactin are thought to be separate hormones in human fetal pituitary (Brauman *et al.*, 1964).

Fetal plasma prolactin levels do not seem to have been reported by previous researchers.

H. Fetal Adrenals, Pancreas, Parathyroid and Thyroid

The fetal adrenal gland is capable of responding to exogenous ACTH, atrophies when the fetal pituitary is removed and is capable of most steroid transformations typical of adult adrenal glands (reviewed by Jost, 1966; Macnaughton, 1969; Pasqualini *et al.*, 1970). Kitchell and Wells (1952) used adrenalectomy and cortisone implantation to establish that a reciprocal relationship exists between the adrenals and hypophysis in the fetal rat.

Bovine fetal adrenals cultured *in vitro* can synthesize carbon-14 corticosteroids from carbon-14 acetate (Chouraqui and Weniger, 1969, 1970). Bassett and Thorburn (1969) detected a six-fold increase of corticosteroid in sheep fetal plasma from 130 days to 150 days of gestation. During this same period maternal plasma corticoids remained constant.

Adrenal medulla hormones have been identified in the bovine fetus (McCord, 1915; Comline and Silver, 1966). Comline and Silver (1966) found that the fetal effluent blood from the adrenal vein contained increased catecholamines in response to anoxia. Thus the evidence indicates that the fetal adrenal gland functions long before birth.

Bovine fetal pancreas was the source of insulin that Banting and Best isolated in 1922. That discovery was possible because the endocrine pancreas become functional before the exocrine pancreas begins to produce proteolytic enzymes. Both insulin and glucagon have been isolated from the fetal pancreas and hyperglycemia elicits insulin release in the fetus (reviewed by Macnaughton, 1969). Several authors have speculated that fetal insulin production in response to hyperglycemia in pregnant diabetic mothers is beneficial in controlling diabetes. However, fetal consumption of glucose may help to control maternal hyperglycemia (Macnaughton, 1969).

Macnaughton (1969) reviewed the fetal parathyroid research and concluded that fetal parathyroid is active in calcium metabolism before birth in many species.

Fetal hypothyroidism appears to be associated with ental deficiencies and retarded ossification (reviewed by ost and Picon, 1970). Hogben and Crew (1923) tried to tablish that a thyroid deficiency in the Dexter cattle

was the cause of the syndrome known as "bulldog dwarf." Their bioassay results did not show a difference between normal and "bulldog" fetuses. Thyroid stimulating hormone (TSH) was found in fetal bovine pituitaries by Bates et al. (1935), who indicated that the developing fetus is autogenously supplied with pituitary hormones. Thyroid hormone injected into fetal rats decreased fetal pituitary TSH, indicating a functional pituitary-thyroid axis in the fetus (reviewed by Jost, 1966; Jost and Picon, 1970).

The first record of bovine fetal serum thyroxine levels was reported by Hernandez (1971) on the same animals used in this thesis study. According to Hernandez, maternal thyroxine levels increase about 19% during gestation while fetal thyroxine level increases from 2.18, 11.66 and 17.16 g/100 ml for 90, 180, and 260 days of age, respectively. The thyroid increased in size in direct proportion to fetal body weight. Females had larger thyroids and more serum thyroxine than males at 180 days of gestation (Hernandez, 1971). Thus, bovine fetal thyroid appears to be metabolically active.

I. Neonatal Serum LH, GH and Prolactin

Macmillan and Hafs (1968) reported LH plasma levels of 0.48 µg/liter in male calves less than 7 days old, and an average LH pituitary concentration of 0.76 µg/ml. Female calves less than 7 days old had average pituitary LH concentrations of 2.44 µg/ml (Desjardins and Hafs, 1968).

Foster (1971) used RIA to quantify LH in neonatal lambs; males had serum LH levels less than 0.3 ng/ml and females less than 0.5 ng/ml during the first 7 postnatal days. Serum LH levels in female lambs increased to 3 ng/ml by day 18 postnatally, while the male lamb showed no increase to day 18.

Neonatal serum GH averaged 33.5 ng/ml in umbilical blood from humans at birth (Grumbach et al., 1968). Purchas et al. (1970) reported that serum GH averaged 32 ng/ml in bulls less than 7 days old using RIA techniques. The pituitaries from the same bulls as above had pituitary GH concentrations of 6 µg/mg by bioassay and 2 µg/mg by RIA (Purchas et al., 1970).

Serum prolactin values for bovine neonates do not appear to be available, however, Reece and Turner (1937) reported prolactin values of 111.4 bird units per calf pituitary and Lyons (1937) found prolactin activity in urine from babies less than 7 days old.

MATERIAL AND METHODS

A. Experimental Design

This research project was designed to study changes in hormone levels and physical changes in the reproductive organs of the bovine fetus during development. These developmental changes were then used to study interactions of maternal and fetal endocrine systems during gestation. A third phase of this project was a study of changes in the bovine endocrine system which occur during the week following birth.

For the purposes of these studies the gestation period was divided into trimesters and a minimum of five male and five female fetuses were collected near the end of each trimester. This sampling procedure permitted statistical analysis of the fetal age and sex differences.

In addition, blood samples were taken from ten calves at birth and daily for one week, to determine hormones during the early neonatal period.

B. Experimental Animals

1. Fetal

Pregnant primiparous Holsteins, 18 to 26 months old, were purchased and transported to Michigan State University. Date of conception was determined by dates of

natural breeding or artificial insemination and verified by rectal palpation. Rectal palpation of the pregnant uteri were performed when the heifers were 25 to 45 days pregnant. Independent estimations of day of pregnancy were made on each heifer by the investigator and by another veterinarian, and then the two estimates were averaged. If this value was not within three days from the reported breeding date for the heifer she was not used in this study. Animals without breeding dates were used if the individual estimates of the length of pregnancy were within five days of each other. Rectal palpation of the embryonic vesicle has proved to be an accurate method of determining length of pregnancy from 28 to 45 days after conception.

At Michigan State University, the animals were maintained on pasture until two or three days before scheduled surgery when they were moved into the Dairy Barn and housed in stanchions. At this time, each animal was palpated to determine if the pregnancy had developed normally and to determine which uterine horn contained the fetus. Eighteen to twenty hours before hysterotomy, each animal was transported to the Veterinary Clinic and placed in a box stall where feed was restricted until after surgery.

After surgery, the heifers were housed overnight the Veterinary Clinic. They were returned to the dairy rn the following day. Postoperative observation was connued for 7 to 10 days.

2. Neonatal

The calves used for the neonatal study were from primiparous Holsteins that had been artificially inseminated with semen from purebred Holstein sires. The pregnant heifers were observed every 2 to 3 hours as parturition approached. Blood samples were drawn from the jugular vein of the calves within 2 hours of birth and daily thereafter for 6 days.

C. Surgery

Forty surgeries were performed. During the surgery the pregnant heifer was restrained in stocks. A 40-ml blood sample was drawn from the jugular vein immediately after the animal was in stocks just prior to surgery. Either the left or right paralumbar area nearest the fetus was prepared for surgery by clipping the hair and scrubbing the area three times with a solution of Betadine.

The selected paralumbar fossa was anesthetized with Procaine (Bio-centic Laboratories, St. Joseph, Missouri). The local anesthetic block for the incision within the paralumbar fossa extended 10 cm parallel to the vertebral lumbar transverse processes on the dorsal margin and 12 cm vertically on the anterior margin of the paralumbar fossa. A 16- to 30-cm incision was made in the abdominal wall in e anesthetized region.

Both the middle uterine artery and vein were cated in the abdominal cavity by palpation and theterized with a polyethylene 30-inch catheter

(Standard Minicath, Desert Pharmaceutical Co., Inc., Sandy, Utah) attached to a 19 gauge x 1.25 inch needle. Forty ml arterial and venous blood samples were drawn using 50 ml syringes attached to the catheters. In some cases difficulty was experienced with catheterization of the middle uterine vein and venous samples were obtained from large veins on the uterine surface. The average elapsed time between the collection of the sample from the jugular vein and the uterine vein was 68 minutes and for the uterine artery 71 minutes.

After collection of the uterine blood the gravid uterine horn was manipulated into the abdominal wall incision and held in place with two pair of vusullem forceps. A 10- to 25-cm incision was made in the greater curvature of the gravid horn with a scissors. The 260-day fetal blood samples were obtained by catheterization of the umbilical vessels with the fetus remaining in utero. The connective tissue covering of the umbilical cord was dissected bluntly with scissors, exposing the four blood vessels. Umbilical arteries and veins could be identified readily by the definite pulse in the former. The smaller 180-day fetuses were removed from the uterus, leaving the placental attachment intact. While the fetus was held aside the cow, the umbilical vessels were catheterized in the same manner described for the 260-day fetuses.

Obtaining adequate blood samples from the relatively small 90-day fetuses proved difficult. The fetus can easily be removed from the uterus leaving the placental attachment functional. Catheterization of the umbilical cord vessels, however, yielded only 1 or 2 ml of blood. Cardiac puncture or decapitation also provided insufficient volumes of blood. Opening the fetal thoracic cavity and catheterizing the thoracic aorta permitted the collection of 10 to 20 ml of blood from the 90-day fetuses.

The average time between the collection of the maternal jugular veinous blood until the umbilical vein and artery were sampled was 73 and 77 minutes respectively. The 90-day fetuses were more difficult and required an average of 90 minutes between collection of the maternal jugular and fetal blood samples.

In all cases, after the fetal blood samples were taken the fetus was removed from the uterus (260 day) and the umbilical cord severed. The uterine incision was inverted and closed with a double row of sutures (Chromic catgut #1) using a Cushing pattern and the uterus was then replaced in normal position. The peritoneum, abdominal muscle layers and subcutaneous tissues were closed separately in layers using a continuous suture pattern (Chromic catgut #2). A synthetic suture (Vetafil Bengen, Haver Lockhart, Kansas City, Missouri) in a continuous lock stitch pattern was used to close the skin incision.

The postoperative care was minimal. More than half of the animals sloughed the placental membranes by seven days after surgery. Otherwise antibiotic infusion of the uterus was necessary to prevent septicemia. Fourteen days after surgery, all placental membranes had been sloughed. Skin sutures were removed 10-14 days after surgery and the animals were turned out to pasture.

D. Blood Serum Samples

All blood samples, except the 90-day fetal samples, were put into centrifuge tubes containing oxalate (31.7 mg) and kept on ice. The blood was centrifuged in a cold room (4°C) within 30 minutes of collection. After centrifugation the plasma was transferred to another centrifuge tube containing $CaCl_2$ (27.8 mg) and stored 24 to 48 hours at 4°C. The samples were then centrifuged to remove the fibrin precipitate, and the serum was transferred to a 20-dram plastic vial and stored at -20°C. Because of variable volumes, the 90-fetal blood samples were collected with oxalated catheters and syringes and transferred to non-oxalated centrifuge tubes. The amount of $CaCl_2$ was adjusted to the sample volume and processed identically as described above.

E. Fetal Tissue Samples

The fetus was weighed and crown-rump length measured within five minutes of removal from the uterus. The

anterior pituitaries were removed after decapitation. Each pituitary was bisected into right and left hemipituitaries. One half was put into Bouins fixative for histochemical studies. The whole pituitary was not weighed because it was necessary to bisect it *in situ* for histochemical studies by Dr. B. Baker, University of Michigan, and these studies will be reported in a subsequent publication.

The remaining hemipituitaries from 90-day fetuses were stored at -20°C for hormone assay. Two or three central slices were removed from the 180- and 260-day hemipituitaries before storage at -20°C. The total weight of these slices, which were used for the *in vitro* incubations, averaged 2.7 mg per pituitary.

Gonads, adrenals, uteri and seminal vesicles were removed and weighed. An average of 20 minutes elapsed between severing the umbilical cord and complete processing of all fetal samples. The gonads from each fetus were sectioned for electron microscopic study by Dr. R. Saacke, Virginia Polytechnical Institute, and also incubated *in vitro* for hormone metabolic studies, both to be reported in subsequent publications.

F. Pituitary Tissue Culture

Tissue culture requires chemically clean specialized equipment and strict asceptic technique. The method used is described in detail below.

1. Equipment

Stainless steel incubation platforms and all glassware were initially washed in Alconox detergent (Alconox, Inc., New York, N. Y.) followed by ten rinses in tap water. Thereafter materials were cleaned with a tissue culture detergent (Micro-Solv, Microbiological Associates, Inc., Bethesda, Maryland) followed by three distilled water rinses. Water used for rinsing of glassware and other materials was distilled over glass and deionized.

All glassware and other equipment were sterilized by autoclaving at 15 psi for 30 minutes. Sterile equipment was transferred to a sterile glove box (Germfree Laboratories, Inc., Miami, Florida) where they were stored until used. This chamber was also used for pituitary dissection and transfer of slices to the culture vessels. An ultraviolet light on the ceiling of the chamber was turned on 30 minutes prior to use to aid in ascepsis.

Surgical instruments for sterile dissection were stored in 70% ethanol. Similarly, 70% ethanol was used to disinfect the dissection areas and the transfer chamber floor.

2. Culture Medium

The medium used for the culture was TC 199 (Difco, Detroit, Michigan). This was purchased as a ten-fold concentrate and prepared in 25-ml quantities for culture;

2.5 ml of concentrated TC Medium 199, 1.0 ml Penicillin-G-Phosphate (1.7 mg/ml), 2.0 ml 2.8% $NaHCO_3$, and 19.5 ml deionized water.

3. Culture Method

Disposable, sterile organ culture dishes with absorbent rings, measuring 60 x 15 mm (Falcon Plastics, Inc., Los Angeles, California) were used for anterior pituitary tissue culture. Stainless steel screen platforms (15 x 15 mm) were placed into these dishes. Lens paper wicks were placed on the screen platforms. One ml of TC 199 culture medium placed in the center well just covered the top of the platform. By this arrangement, pituitary explants were exposed to the gaseous environment yet received adequate nutrition from the culture medium.

The gas environment for all cultures consisted of 95% O_2 and 5% CO_2. Gassing was continuous at a flow rate of 300 ml/minute. Humidification of the gas was accomplished by bubbling it through sintered glass filters submerged in distilled water before entry into the culture chamber. Explants were incubated in a culture oven for 72 hours at 37°C. At this time the explants and medium were separately recovered and stored at -20°C until assayed.

G. Pituitary Homogenization

In preparation for hormone assay, pituitaries were minced on a wax block, placed in a test tube containing

2 ml of phosphate buffered saline (PBS) (Appendix I.B.1) and homogenized by sonication (Sonifier Cell Disruptor, Model W185D, Heat Systems-Ultrasonics, Inc., Plainview, New York). The tube containing the pituitary slices was immersed in ice water during sonication to prevent overheating. Sixty to eighty watts were applied intermittently for a total of three minutes. The anterior pituitary slices obtained from the incubation procedure described above were processed in the same manner. All pituitary homogenates were stored at -20°C until assayed.

H. Radioimmunoassay (RIA)

1. Luteinizing Hormone (LH)

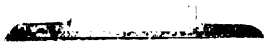
The bovine LH assay was similar to the assay reported by Niswender et al. (1969). LH antibody was developed by repeated injections of NIH-LH-B5 into guinea pigs (Appendix I.C.1). Purified bovine LH used for iodination (LER-1072-2) was supplied by Dr. Leo Reichert (Emroy University, Atlanta, Georgia). This preparation had an LH potency of 1.66 NIH-LH-SI units/mg and showed no FSH activity when tested at 3600 µg in the Steelman-Pohley assay. It had a thyroid stimulating hormone (TSH) contamination estimated at 0.021 USP units/mg.

a. Radioiodination.--Purified bovine LH (LER-1072-2) had been previously dispensed into 1-ml vials (2.5 µl of a 1 µg/ul solution in glass distilled water) and stored at -20°C. These vials were thawed immediately before iodination and the iodination procedure was performed at room

temperature. Twenty-five µl of 0.5 M sodium phosphate buffer at pH 7.5 (Appendix I.A.1) was added to the hormone and mixed. One mCi of ^{125}I (50 mCi/ml, Iso-Serve Division of Cambridge Nuclear Corporation, Cambridge, Massachusetts) was added, and the contents gently mixed.

Forty µg cloramine-T (Eastman Organic Chemicals, Rochester, New York) (Appendix I.A.3) was added to the vial, the vial was stoppered, and the contents were gently mixed by finger tapping. The reaction was stopped at exactly two minutes by adding 125 µg sodium metabisulfite (Appendix I.A.4). After thorough mixing, 25 µl of 2.5% bovine serum albumin (BSA, Nutritional Biochemicals, Inc., Cleveland, Ohio) in 0.01 M phosphate buffered saline (PBS) pH 7.0 (PBS-2.5% BSA) was added to diminish the loss of hormone adhering to the glass vial.

A 1 x 12 cm glass column packed with Bio Gel P-60 (Bio Rad Labs, Richmond, California) was equilibrated previously by passing 0.05 M sodium phosphate buffer pH 7.5 (Appendix I.A.2) through, and then 2 ml PBS-2.5% BSA was added and eluted with buffer to reduce non-specific binding of the protein hormone to glass. One hundred µl of transfer solution (Appendix I.A.5) was added to the vial with iodinated LH and the contents of the vial were layered beneath the buffer on the surface of the column. Seventy µl of rinse solution (Appendix I.A.6) was added to the hormone vial, recovered, and also layered beneath the



buffer on the column. The iodinated LH was eluted from the column under gravity with 0.05 M sodium phosphate buffer and 15 1 ml aliquots were collected from the column in 12 x 75 mm disposable glass tubes containing 1 ml of 2% lyophilized egg white albumin (EWA, Sigma Chemicals Co., St. Louis, Missouri) in PBS (PBS-2% EWA). The elution profile was determined by quantifying the radioactivity of 10 µl from each of the 15 tubes in an automatic gamma counter (Nuclear Chicago Corp., Des Plaines, Illinois).

As an example of an elution curve (Figure 1), the first peak represents iodinated LH and the second peak represents free ^{125}I. The peak ^{125}I-LH tube was used in the LH RIA. The iodinated LH was quite stable and when stored at -40°C, could be used up to 1 month after preparation. When used after this time, the free ^{125}I and radioation-damaged ^{125}I-LH which develop during storage could be then separated on a 1 x 12 cm column of Sephadex G-100 (Pharmacia Fine Chemicals Inc., Piscataway, New Jersey) eluted with 0.05 M phosphate buffer (pH 7.0). A typical elution pattern to repurify ^{125}I-LH had three peaks of radioactivity. The first and second peaks represented iodinated LH, the first peak was damaged LH as indicated by the fact that when an equal number of cpm from peaks one and two were incubated with anti-LH, more activity from peak two was bound. The third peak represented free ^{125}I.

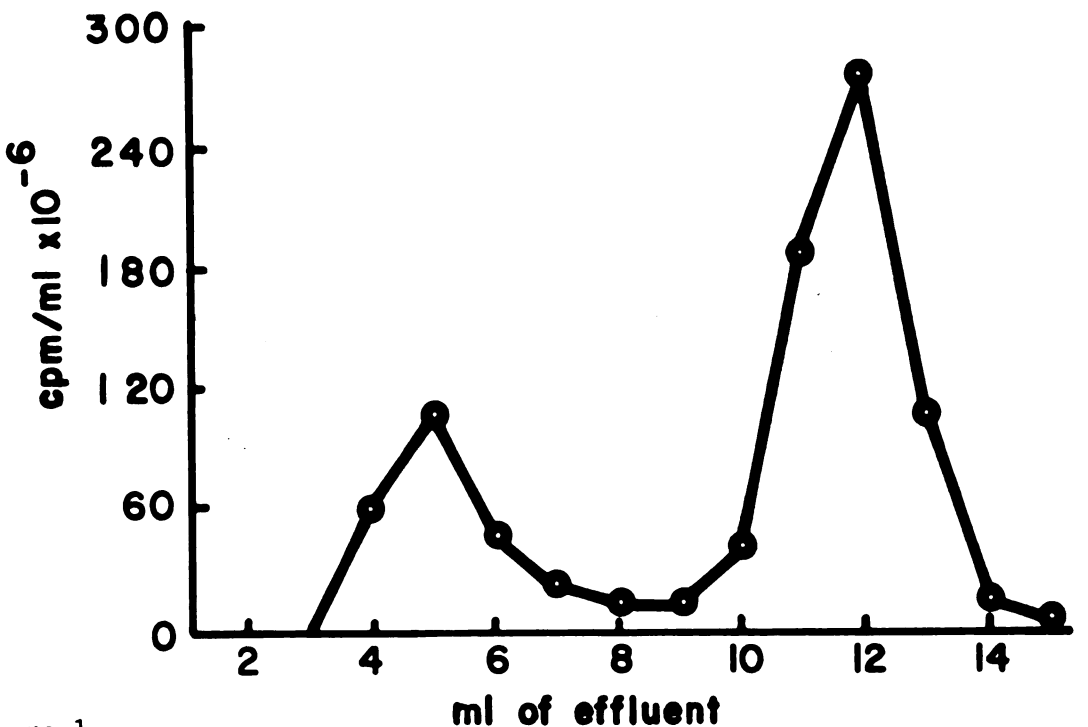


Figure 1

Elution profile of iodinated luteinizing hormone (LH) after passage through Bio - Gel P-60. The first peak represents iodinated LH and the second peak represents free iodine.

b. Radioimmunoassay.--Each unknown (serum, pituitary homogenate or *in vitro* incubation medium) was assayed in dilution duplicate. Two selected dilutions made in PBS-1% EWA (Appendix I.B.3) of each unknown were added to separate disposable glass culture tubes (12 x 75 mm) with a Hamilton microliter syringe (Hamilton Co., Whittier, California). PBS-1% EWA (Appendix I.B.3) was then added to give a total of 500 µl. Pituitary homogenates and culture media were diluted up to 1:10,000 in PBS-1% EWA to center within the standard LH curve. As discussed by Hunter (1967), use of two dilutions provides evidence of the specificity of the assay which is not provided with duplicate determinations of the same dilution. Each lot of 100 tubes included 10 tubes containing 0, 0.08, 0.16, 0.32, 0.64, 1.28, 2.56, 5.12, 10.24, and 20.48 ng of standard LH (NIH-LH-B5, National Institutes of Health, Endocrinology Study Section, Bethesda, Maryland) (Appendix I.B.4).

Two hundred µl of LH antibody (Appendix I.B.6), hereafter referred to as first antibody, was added at a dilution of 1:200,000 to each of the culture tubes and the tubes were incubated at 4°C for 24 hours. Solutions of ^{125}I-LH for RIA were prepared by diluting the stock ^{125}I-LH with PBS-1% EWA so that 100 µl contained about 20,000 CPM. One hundred µl of ^{125}I-LH solution was then added to each tube. Incubation was continued at 4°C for 24 hours.

Goat anti-guinea pig gamma globin (GAGPGG, Appendix I.C.2), later referred to as the second antibody, was diluted (Appendix I.B.7) to a titer which would optimally precipitate the gamma globulin. The second antibody formed an antigen-antibody-antibody complex large enough to be precipitated by centrifugation. Two hundred µl of GAGPGG was added to each tube and incubation continued for 72 hours. After each addition, the tubes were vortexed gently and covered during the incubation to retard evaporation.

Following the final incubation, 3 ml of cold PBS (Appendix I.B.1) was added to each tube to dilute the unbound ^{125}I-LH. The bound ^{125}I-LH was precipitated by centifugation at 2500 x g for 30 minutes in a refrigerated centrifuge with a swinging bucket rotor (Sorvall Model RC-3, Ivan, Sorvall, Inc., Norwalk, Connecticut). The supernatant fluid was decanted and the tubes allowed to drain for 30 minutes and remaining fluid adherent to the neck and lip of the tube was removed with absorbent tissue. The bound ^{125}I-LH of the precipitate was then quantified in an automatic gamma counter. Samples were usually counted for 10 minutes or for a total of 10,000 counts, whichever accumulated first. This information was punched automatically on paper tape by a Teletypewriter (Teletype Corp., Skokie, Illinois). The standard curve was calculated by multiple regression analysis on a CDC 3600 computer.

The values for standard LH assay fit linear, quadriatic and cubic components of the regression equation: correlation coefficients were consistently 0.99 to 1.00. These regression coefficients were entered manually into an Olivetti computer (Programma 101, Olivetti Underwood, New York, New York), the counting time for each unknown was entered into the computer from the punched tape through a Punched Tape Editor (Beckman Model 6912 Tape Editor, Beckman Instruments, Inc., Fullerton, California), and LH concentrations in the unknowns were computed automatically.

Control tubes were included in each assay to determine background radioactivity (tube containing 1:400 control guinea pig serum in place of the first antibody), total counts added (tube containing only ^{125}I-LH) and counts in the precipitate (tube containing no unknown or standard). Values for the duplicate standards were averaged and plotted as the percent of ^{125}I-LH precipitated at each dose of LH standard (Figure 2).

c. Selection of Assay Conditions.--Yalow and Berson (1968) stated that maximum sensitivity of the assay was obtained when at least one-third of the labeled hormone is bound. Because the binding activity varied for the different iodinated LH preparations, several dilutions of the first antibody were used in the assay system after each new iodination to insure use of the proper dilution. But it usually was diluted initially to 1:200,000; giving a

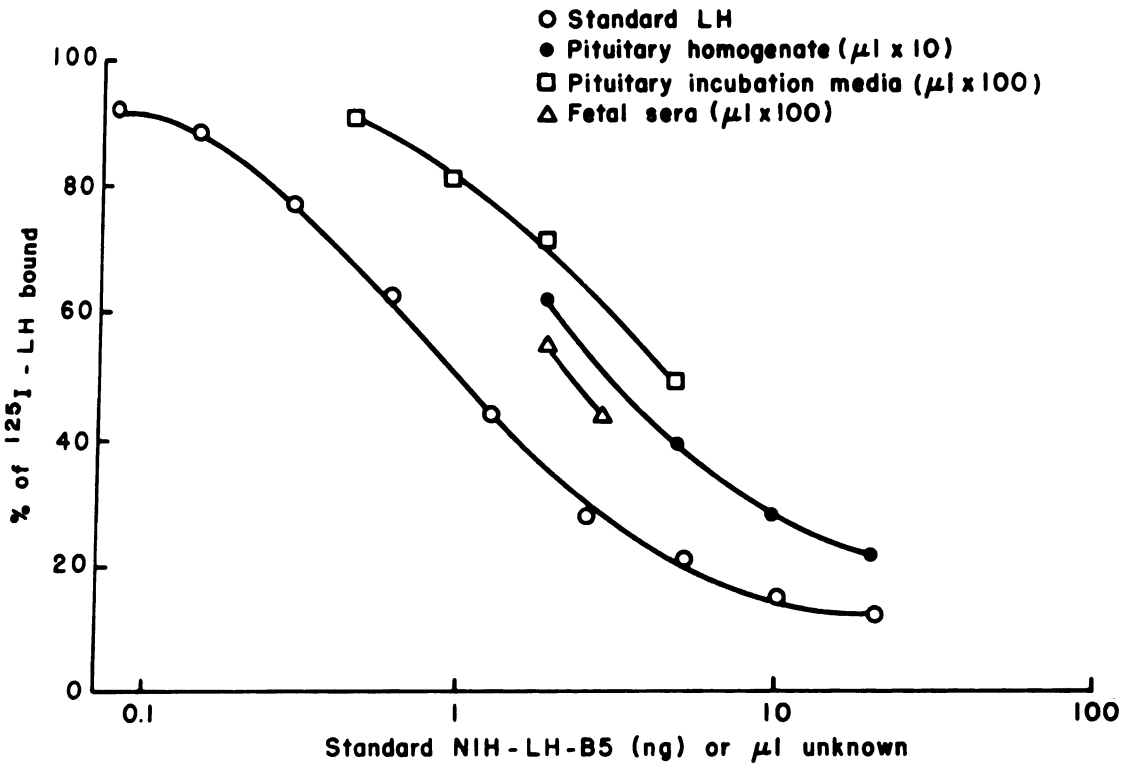


Figure 2 Dose response curves for NIH-B5-LH standards and for bovine fetal sera, pituitary homogenates and pituitary incubation media.

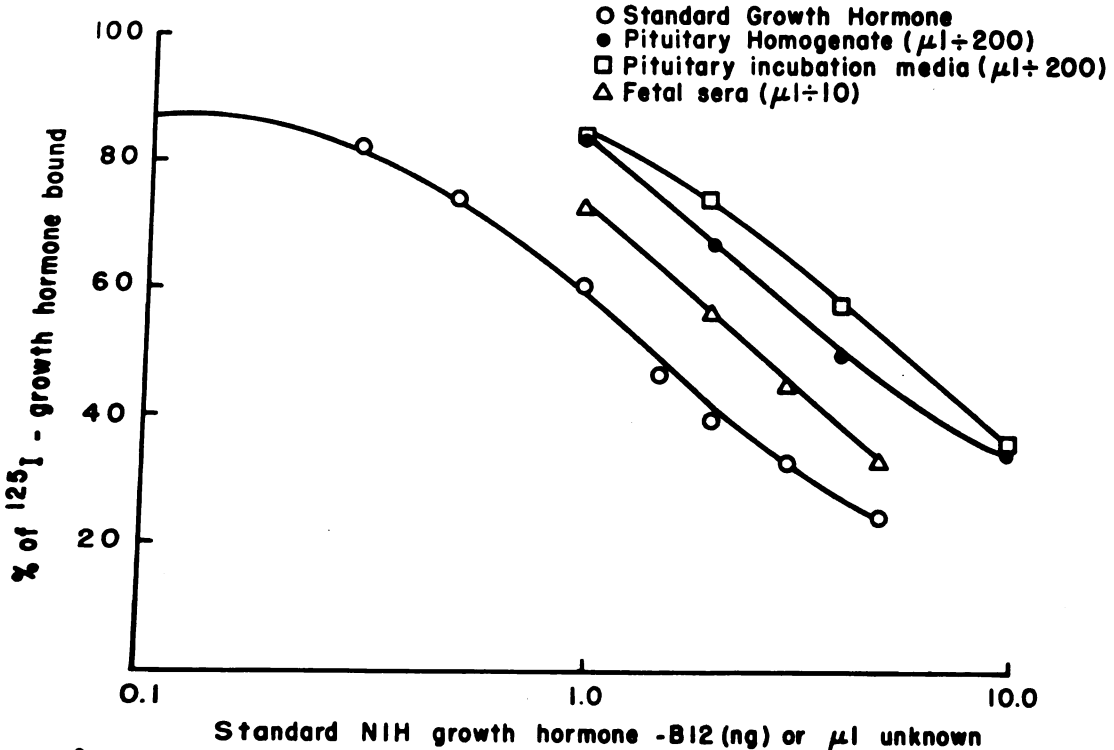


Figure 3 Dose response curves for NIH-GH-B12 standards and for bovine fetal sera, pituitary homogenates and pituitary incubation media.

final dilution of 1:1,000,000 after all the reagents had been added. First antibody produced an average of 35% binding of iodinated LH at the 1:200,000 dilution. The second antibody was always added at a dilution (usually 1:5 or 1:6) which yielded maximum precipitation.

d. Validation of the Assay.--The high dilution of the first antibody resulted in excellent sensitivity and it was possible to detect as low as 0.30 ng LH/ml serum. Specific amounts of NIH-LH-B5 were added to tubes containing 100 µl serum to establish the accuracy. Precision was reduced when more than 2 ng exogenous LH was added (Table 1), but adequate at lower levels comparable to those found in serum.

TABLE 1.--Recovery of NIH-LH-B5 from 100 µl serum.

LH added	Serum LH Recovery		
	1	2	Average
--(ng)--	----------(ng/ml)----------		
0.12	0.10	0.08	0.09
0.50	0.46	0.36	0.41
2.00	1.65	2.22	1.93
8.20	4.80	12.00	8.40

Preparations representing high levels of follicle stimulating hormone (FSH), growth hormone (GH), prolactin or thyroid stimulating hormone (TSH) relative to the level of LH did not appear to affect the ability of the antisera to selectively bind LH.

Dilutions (1:10 to 1:10,000) of the TC-199 media used for the pituitary incubations were tested for binding activity with the antisera. No inhibition of ^{125}I-LH binding was observed.

Estimates of LH in different dilutions of fetal bovine serum, pituitary homogenates and incubation medium were consistent and the slope of the curve was parallel to the curve for standard LH (Figure 2). Thus the assay appeared very sensitive and specific, and ideally suited to quantifying bovine fetal LH.

2. Growth Hormone (GH)

The procedure used to quantify bovine fetal GH was developed by Purchas (Purchas et al., 1970). Anti-bovine GH (GPABGH) was developed in guinea pigs by repeated subcutaneous injections of (NIH-GH-B12).

The second antibody, sheep anti-guinea pig gamma globulin (SAGPGG) was developed similarly to the (GAGPGG, Appendix I.C.2).

a. Radioiodination.--The procedure to iodinate GH was similar to that described for LH with the exception of the following variations.

Five µg of NIH-GH-B12 was reacted with ^{125}I-GH for 2 minutes in the presence of 75 µg chloramine-T. Other procedures were similar to the LH iodination with the exception that 1 ml aliquots from the Bio Gel P-60 column were collected in tubes containing 1 ml PBS-2% BSA.

^{125}I-GH could be used for two or three weeks after iodination before repurification on Sephadex G-100 was required.

b. Radioimmunoassay.--Because of high levels of GH in some samples, it was necessary to dilute from 1:2 to 1:30,000 with PBS-1% BSA (Appendix I.B.3). This buffer was used in the GH RIA. The diluted unknowns were then assayed in dilution duplicate. Each lot of 100 assay tubes included 10 tubes containing 0.1, 0.3, 0.5, 0.8, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0 ng of standard GH (NIH-GH-B12, Appendix I.B.4). The volumes of first antibody (GPABGH, Appendix I.B.6), second antibody (SAGPGG, Appendix I.B.7) and ^{125}I-GH used and the incubation procedures were identical to those used for the LH assay.

c. Selection of Assay Conditions.--A 1:3,200 dilution of GPABGH was used as the binding antibody. Precipitation of antibody-bound GH from free GH was accomplished by adding a 1:3 to 1:5 dilution of SAGPGG. Incubation intervals were identical to those in the LH assay.

d. Validation of Assay.--Estimates of GH in varying amounts of bovine fetal serum, pituitary homogenates and incubation medium were internally consistent and the slope of the curve was parallel to the curve for standard GH (Figure 3, p. 52). Recovery of standard GH and cross reactions were discussed by Purchas et al. (1970). This assay appeared to be suitable for quantifying bovine fetal GH.

3. Prolactin (P)

The procedure to quantify bovine fetal prolactin was developed by Tucker (1971). Anti-bovine prolactin (GPABP) was developed in guinea pigs by repeated subcutaneous injections of NIH-P-B1 in the scapular region.

The second antibody (SAGPGG) used for the prolactin assay was the same as that described for the GH assay.

a. Radioiodination.--The procedure to iodinate prolactin was nearly identical to GH, except that 5 µg of NIH-P-B1 was reacted with ^{125}I for 2 minutes in the presence of 25 µg Cholamine-T. Because purified bovine prolactin was not available NIH-P-B1 was used as a source of prolactin for iodination. As discussed by Swanson (1970) prolactin (NIH-P-B1) is not a homogenous protein but homogeneity is not required in the radioimmunoassay.

b. Radioimmunoassay.--The high levels of prolactin in the unknowns required dilutions from 1:2 to 1:10,000 with PBS-1% BSA (Appendix I.B.3). The unknowns were then assayed in dilution duplicates. Each lot of 100 assay tubes included 10 tubes containing 0.1, 0.2, 0.5, 0.8, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0 ng of standard prolactin (NIH-P-B2, Appendix I.B.4). One hundred µl of first antibody (GPABP, Appendix I.B.6) and 100 µl of second antibody (SAGPGG, Appendix I.B.7) were used in the RIA assay for prolactin.

c. Selection of Assay Conditions.--A 1:30,000 dilution of GPABP was used as the binding antibody. Precipitation of antibody bound prolactin from free prolactin was accomplished by adding a 1:3 to 1:5 dilution of SAGPGG. Incubation intervals were identical to those in the LH assay.

d. Validaton of Assay.--A typical standard curve and estimates of prolactin in varying amounts of bovine fetal serum, pituitary homogenates and incubation medium were internally constant and the slope of curves were parallel to the curve obtained with the standards (Figure 4). Recovery of exogenous NIH-P-B1, precision and specificity for this prolactin RIA were discussed by Swanson (1970). The prolactin RIA proved a valid method for quantifying bovine fetal prolactin.

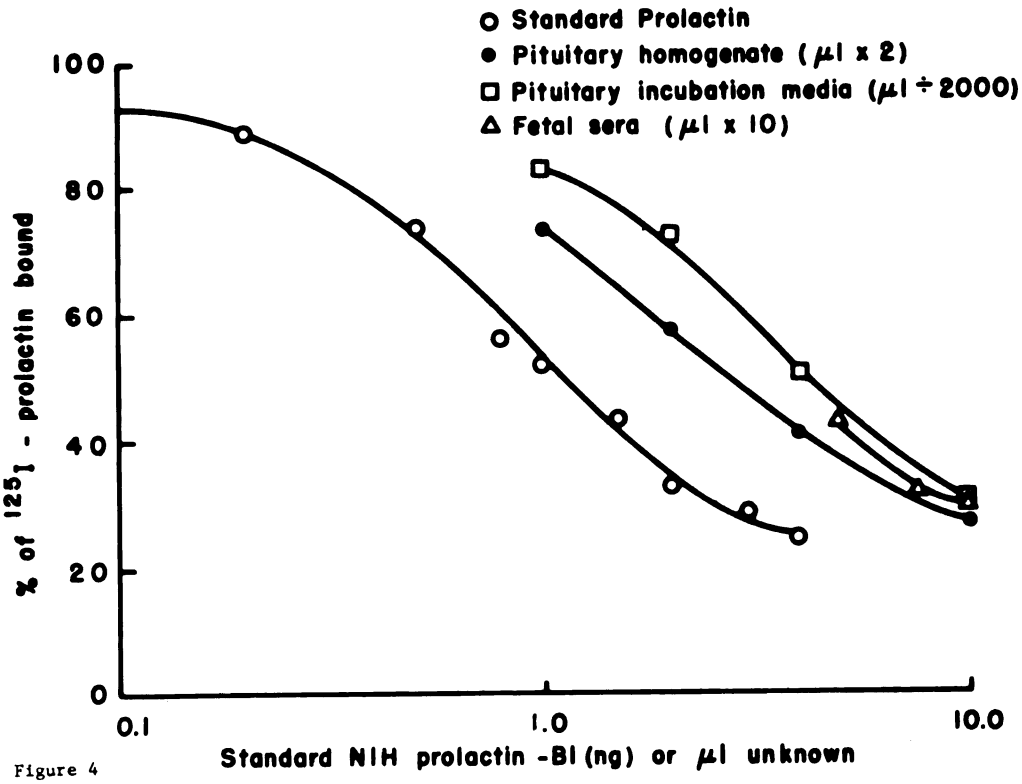


Figure 4

Dose response curves for NIH - BI- prolactin standards and for bovine fetal sera, pituitary homogenates and pituitary incubation media.

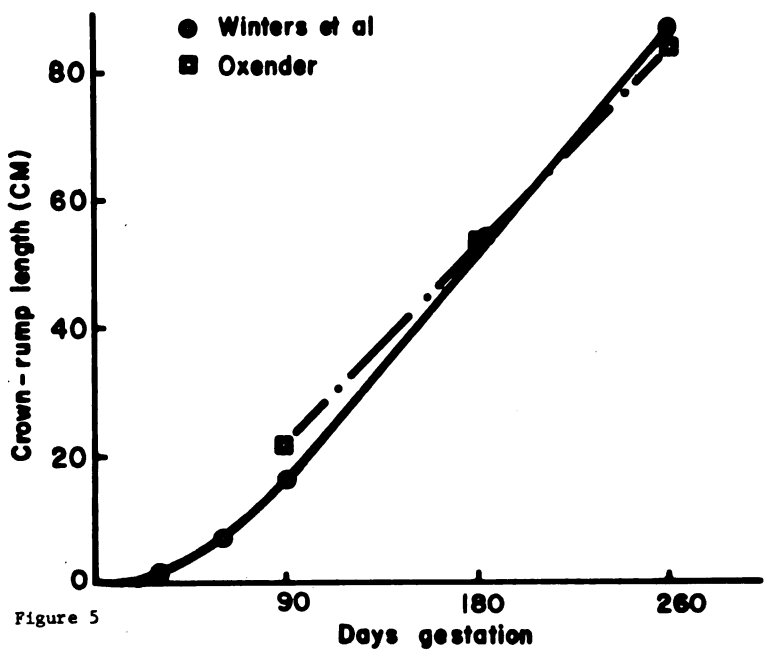


Figure 5

Comparison of Crown - rump length and fetal age

RESULTS AND DISCUSSION

All of the data presented in this section are presented as means and standard error of means. Data for cows and fetuses are listed individually in Appendices II through VIII.

A. Fetal Physical Data

1. Age, Body Weight and Crown-rump (CR) Length

Since this study concerned fetal development, age becomes a critical factor (Jost and Picon, 1970). For this reason, ages of fetuses in this study were carefully established as the number of days elapsed between breeding the cow and surgical collection of the fetus. Fetal ages are listed by sex in Table 2. The age difference between sexes is not large, and hereafter I will use 90-day, 180-day or 260-day fetuses when referring to fetal age.

Growth of the fetus can be expressed in terms of body weight or crown-rump length. From 90 days to 260 days, body weight increased from 0.5 to 26.7 kg (Table 2); more than a 50-fold increase compared with only a 4-fold increase in crown-rump length during this same interval. Normal fetal weight increases exhibit an exponential relationship with fetal age. The 90-day fetuses used in this

TABLE 2.--Fetal body weight, crown-rump length and adrenal gland weight.

Sex	Age	Body weight	Crown-rump length	Adrenal
	(days)	(kg)	(cm)	(mg)
Male	90	0.5±0.1[a]	22.6±1.3	124±30
Female	91	0.4±0.1	22.3±0.6	98±11
Average	91	0.5±0.1	22.5±0.6	108±13
Male	181	6.4±0.4	53.1±1.5	731±45
Female	180	6.5±0.3	55.1±1.2	773±52
Average	180	6.4±0.2	54.0±1.0	750±33
Male	256	27.9±3.6	85.3±4.2	1,788±80
Female	263	25.2±3.7	83.3±4.7	1,648±236
Average	259	26.7±2.5	84.4±3.0	1,724±111

[a]Mean ± standard error, n = 5 to 8.

study appeared larger than those reported in earlier studies. Winters et al. (1942) reported single fetal body weights of 0.16, 6.7 and 31.3 kg at 90, 185 and 260 days of age, respectively. Roberts (1956), in a review of bovine fetal body weights, gave ranges of 0.1-0.2, 5-8 and 25-50 kg for fetal ages of 90, 180 and 270 days, respectively, but the number of animals used to establish these values was not always indicated.

Although the average body weight for the 13 90-day fetuses in this study was higher than those reported previously, the averages for 180- and 260-day fetal body weights were similar to earlier reports. Fetal body weights and age were correlated ($r = 0.86$, $P < 0.01$).

Crown-rump (C-R) length (Table 2) is a more widely used criterion when discussing fetal age-related developments, principally because the exact fetal age usually is unknown. The C-R length of bovine fetuses increased linearily from 90 days to birth (Figure 5, p. 58). The body weights and C-R length of the 13 90-day fetuses averaged appreciably higher than those measured by Winters et al. (1942). The C-R lengths of the 180- and 260-day fetuses were within the ranges quoted by Roberts (1956) and closely paralleled the C-R lengths reported by Winters et al. (1942) and included in Figure 5.

Fetal body weight and C-R length were correlated ($r = 0.93$, $P < 0.01$), but C-R lengths were more highly correlated with fetal ages ($r = 0.97$) than body weights. In this thesis, all further fetal age-related correlations will be reported in terms of C-R length.

Many factors influence fetal body weight. In addition to factors inherited from the sire and dam, the age of the dam, breed and sex of the fetus also influence fetal weight. To control as many sources of variation as possible, the fetuses were all from primiparous Holstein dams and sired by purebred Holsteins.

2. Adrenal and Gonad Weights

Paired fetal adrenal gland weights are shown in Table 2. Sex differences in gland weights were not significant. During the 90-day to 180-day period, the average

adrenal weight increased more than 7-fold while only a 2-fold increase in weight occurred during the last trimester of gestation. Weights of the right (404 mg) and left (410 mg) glands did not differ significantly. Comline and Silver (1966) reported that left fetal adrenals weighed 350 mg in 180-day and 1,110 mg in 260-day Jersey fetuses. Adrenal gland weights were correlated (r = 0.96) with C-R length. The active function of the fetal pituitary-adrenal axis has been verified in several species (reviewed by Jost and Picon, 1970). Bassett and Thorburn (1969) measured fetal adrenal corticoid levels in fetal sheep and found a rapid increase the last 20 days of gestation. Drost and Holm (1968) adrenalectomized sheep fetuses, and reported prolonged gestation in the ewes carrying adrenalectomized fetuses. Several reports including Adams and Wagner (1970) have hypothesized that the fetal adrenal gland actively participates in initiation of parturition.

Fetal paired testes weights (Table 3) averaged 131, 1384 and 4136 mg for 90, 180 and 260-day fetuses, respectively. These testes weights agree with data from Matschke and Erickson (1969) on single testis weights of 40, 520, 2150 mg for 85, 170 and 270 days, respectively. Karg (1967a) also reported similar fetal testes weights. Testes weights were correlated (r = 0.91) with C-R lengths, and with adrenal weights (r = 0.90).

TABLE 3.--Fetal testicular and seminal vesicular weights during gestation.

Organ	Days Gestation 90	180	260
	(mg)		
Testes	131±20[a]	1384±136	4136±597
Seminal vesicles	---	515±103	1236±219

[a]Mean ± standard error, n = 5 to 7.

Fetal ovaries are not as large as fetal testes, a situation also true in adults. Paired ovarian weights (Table 4) averaged 83, 287, and 1027 mg for 90, 180 and 260-day fetuses, respectively, similar to the 52, 264, and 1319 mg for 90, 190 and 270-day fetuses reported by Erickson (1966). Crown-rump lengths were correlated ($r = 0.84$, $P < 0.01$) with ovarian weights. Bovine fetal ovaries are capable of steroid biosynthesis (Roberts and Warren, 1964), but it is not known if prenatal function of ovaries is

TABLE 4.--Fetal ovarian and uterine weights during gestation.

Organ	Days Gestation 90	180	260
	(mg)		
Ovaries	83±6[a]	287±66	1027±222
Uterus	130±13	1975±172	4916±832

[a]Mean ± standard error, n = 5 to 8.

necessary for fetal reproductive development (Macnaughton, 1969). Testicular, ovarian and adrenal weight increases were each significantly ($P < 0.01$) correlated with C-R length increases. The adrenal-ovarian weight correlation ($r = 0.91$) and adrenal-testes weight correlation ($r = 0.90$) indicate that these three fetal endocrine glands develop in similar patterns with increasing fetal age.

3. Uterine and Seminal Vesicular Weights

Fetal uterine weights (Table 4) increased parallel to body development (C-R length vs. uterine weight $r = 0.95$, $P < 0.01$). The uterus is readily identified in the 90-day fetuses, indicating that sexual differentiation of the Mullerian ducts was completed.

In 90-day fetuses the seminal vesicles could be identified macroscopically but the tissues were too soft for accurate dissection of the seminal vesicles, so only the 180- and 260-day weights were recorded (Table 3). The increase in seminal vesicular weight was related to fetal growth ($r = 0.80$, $P < 0.01$). The uterus of the female fetuses appeared more highly developed than the seminal vesicles at 90 days of fetal age. Furthermore, increases in uterine weight during pregnancy were proportionately greater than increases in seminal vesicular weight.

B. Maternal Serum Hormones during Gestation

The body weights of the primiparous Holstein females used for this project are shown in Table 5. The ages of these heifers ranged from 16 to 24 months at surgery, and their body weights were comparable to those given in Morrison's Table of Weights for those ages of Holstein females (Morrison, 1957).

TABLE 5.--Body weight of pregnant heifers.

Days Gestation	Weight
	(kg)
90	373±12[a]
180	385±8
260	449±18

[a]Mean ± standard error, n = 11 to 13.

Three blood samples were taken from each cow during surgery. A sample was drawn from the jugular vein and, in an attempt to determine arterial-venous hormone differences across the uterus (and placenta), one blood sample was collected from the middle uterine artery and another from a uterine vein. Usually, blood was taken from the utero-ovarian vein, but in some cases a collateral vein leading to the utero-ovarian vein was used. Yamauchi and Sasaki (1968, 1969) showed that the middle uterine artery was the major arterial blood supply to the uterus while the utero-ovarian vein was the major vein carrying blood

from the uterus. The uterine blood vessels were identified by palpation for catherization. The middle uterine artery can be easily located in the broad ligament of the uterus. The utero-ovarian vein has tortuous small arteries on its wall which helps to identify it by palpation (Yamuchi and Sasaki, 1969).

The data presented in this section were analyzed statistically in two steps. First an F-test was used to test the hypothesis that the sex of the fetus has no effect on maternal serum hormone levels. This hypothesis had to be rejected since significant sex differences were found. A three-factor analysis of variance was then used to analyze the data for each hormone. The three factors were sex of the fetus, days of gestation and source of the blood sample.

1. Luteinizing Hormone (LH)

It is possible that the maternal serum LH levels were influenced by the sex of her developing fetus; jugular blood from cows with male fetuses averaged 0.10 ng/ml less ($P \sim 0.11$) LH at 180 days gestation than cows with female fetuses (Table 6). Blood serum LH values for the cows (Table 7) ranged from 0.5 to 1 ng/ml, and did not differ significantly at the three stages of gestation. In all cases, the LH level of the uterine artery was higher than that of the uterine vein, but this difference was not significant and suggests that placental production,

TABLE 6.--Jugular blood serum growth hormone (GH), LH and prolactin in cows carrying male or female fetuses.

Hormone	Sex	Days Gestation 90	180	260
		(ng/ml)		
GH	Male	6.0±1.8[a]	10.8±4.2	12.3±4.7
	Female	5.5±1.7	4.4±0.9	7.3±5.0
LH	Male	0.82±0.08	0.80±0.07	0.75±0.07
	Female	0.83±0.05	0.90±0.08	0.74±0.07
Prolactin	Male	378±280	196±134	596±295
	Female	120±66	85±43	87±33

[a]Mean ± standard error, n = 5 to 8.

TABLE 7.--Blood serum LH in the cow during gestation.

Source	Days Gestation 90	180	260
	(ng/ml)		
Number of cows	13	13	11
Jugular vein	0.82±0.04[a]	0.85±0.05	0.74±0.05
Uterine artery	0.89±0.07	0.85±0.10	0.85±0.10
Uterine vein	0.84±0.04	0.78±0.03	0.74±0.04

[a]Mean ± standard error.

transfer or uptake of LH was small. Schams (1969) measured LH in two cows three times daily during the first 2 months of gestation, and found consistently low LH levels (< 1 ng/ml) except for two unusually high values. Jaffe et al. (1969) found low LH levels in a pregnant woman and theorized that the LH activity of HCG may suppress LH release during pregnancy. Since LH is considered the major luteotropic hormone in the bovine, increases in the level

of LH would be expected during increased progesterone output during pregnancy (Stabenfeldt et al., 1969). The bovine placenta, however, may be one source of the progesterone during gestation (Ainsworth and Ryan, 1967), especially during the third trimester when pregnancy may proceed in the absence of the ovaries (Amoroso and Finn, 1962).

Serum LH increases and decreases rapidly near estrus (Swanson, 1970). The high LH level during the ovulatory peak lasts for only a few hours. The sampling procedure in this study was not designed to detect short duration LH changes during gestation.

2. Growth Hormone (GH)

An interesting observation concerning maternal serum GH levels is shown in Table 6. The serum GH was more than 68% higher in cows with male fetuses ($P \sim 0.11$) than in cows with female fetuses. Since GH does not appear to be transferred readily by the placenta (Gitlin et al., 1965), the maternal increase in GH serum levels may have resulted from indirect maternal pituitary stimuli that originated in the male fetus. For example, androgens may be transferred by the placenta (Shane et al., 1969; Resko, 1970). In any case, knowledge of the function of maternal serum GH during gestation is incomplete.

Jugular serum GH in the cow during gestation (Table 8) increased gradually from 5.7 to 10.1 ng/ml during gestation, but this increase was not significant. In

TABLE 8.--Blood serum growth hormone in the cow during gestation.

Source	Days Gestation 90	180	260
	----------(ng/ml)----------		
Number of cows	13	13	11
Jugular vein	5.7±1.3 [a]	7.8±2.4	10.1±3.3
Uterine artery	2.2±0.3	3.0±0.6	1.9±0.1
Uterine vein	1.8±0.2	2.5±0.2	1.8±0.2

[a]Mean ± standard error.

contrast to LH, GH in the jugular serum was 100 to 500% higher than that in the uterine artery or uterine vein ($P < 0.01$). As with LH, the uterine artery levels of GH were higher than the uterine vein samples, but here again the difference was not statistically significant suggesting little placental production, transfer or uptake of GH.

Grumbach et al. (1968) found serum GH in pregnant women averaged 7 ng/ml and did not change during gestation. The cow GH serum levels in jugular samples ranged from 5.7 to 10.1 ng/ml, similar to those for humans.

The reason that the uterine blood serum levels averaged only 20 to 50% of the GH in the jugular vein remains unexplained. A possible explanation may be rapid serum degradation or selective absorption of serum GH. The anterior pituitary releases GH into the venous system which empties directly into the jugular vein. One could expect maximum concentrations of all pituitary hormones

including GH in the jugular vein. Another possible factor contributing to the large decrease in GH in uterine vessels compared with jugular GH could be the time that elapsed between collecting the two samples. The uterine samples were taken approximately 1 hour after the jugular samples.

3. Prolactin

As indicated previously for GH and LH, the sex of the fetus appears to influence maternal levels of prolactin (Table 6). The average serum prolactin (jugular vein) for cows with male fetuses was 596 ng/ml, significantly higher ($P < 0.05$) than the 87 ng/ml average for cows with female fetuses. Even though considerable overlap existed in the prolactin data from individual cows with male or female fetuses, it appears that the male fetus is responsible for the higher maternal serum prolactin.

Maternal serum prolactin levels increased as the gestation period lengthened (Table 9). The jugular vein

TABLE 9.--Blood serum prolactin in the cow during gestation.

Source	Days Gestation		
	90	180	260
	(ng/ml)		
Number of cows	13	13	11
Jugular vein	220±114[a]	145±74	365±174
Uterine artery	159±54	171±52	207±70
Uterine vein	96±29	118±40	153±41

[a]Mean ± standard error.

serum prolactin increased more than 65% from 90 to 260 days of gestation (220 to 365 ng/ml, respectively). Uterine artery prolactin was slightly higher than in the uterine vein at each trimester of gestation. Both uterine artery and uterine vein serum prolactin were lower than the jugular vein levels ($P \sim 0.13$). The explanation for the higher serum prolactin in the jugular vein serum resemble the explanations previously advanced for GH; there was no evidence for placental production of prolactin in maternal blood. The average serum prolactin ranged from 96 to 365 ng/ml depending on the source of serum and stage of gestation.

Raud et al. (1971) reported that, under controlled conditions, the serum prolactin averaged 31 to 64 ng/ml in nonpregnant cows with considerable baseline variation among individual cows, and increased up to 400 ng/ml after the animals were stressed. Values up to 880 ng/ml of serum prolactin in cows 1 day prepartum were reported by Schams and Karg (1970). In several stress studies, Raud et al. (1971) observed relatively rapid increases in serum prolactin, which gradually declined to basal levels in approximately 4 hours. Physiological changes in prolactin levels may be masked by the stress of sample collection unless indwelling catheters are used. Only in this way can release of prolactin due to stress be minimized. Other factors which could have contributed to stress release of prolactin were hysterotomy and trucking of the animals to the

veterinary clinic before surgery. In the present study, some prolactin release due to stress may be confounded with the changes apparently due to gestation.

Prolactin may aid in mammary gland development during gestation (Lyons, 1958), but the present results suggest prolactin may have other implications during gestation in the cow.

C. Fetal Pituitary Hormones

1. Pituitary Content

The data for fetal pituitary hormone content was analyzed statistically in a two-factor analysis of variance, fetal sex and age constituted the two factors. The three ages were compared with orthogonal contrasts.

a. Luteinizing Hormone (LH).--Fetal pituitary LH levels increased from 323, to 474, and to 535 ng/mg anterior pituitary for 90, 180 and 260-day fetuses, respectively ($P \sim 0.07$), Table 10). The increase ($P < 0.01$) in pituitary LH from

TABLE 10.--Fetal anterior pituitary LH content.

Sex	Days Gestation		
	90	180	260
	---------- (ng/mg) ----------		
Male	285±93[a]	413±74	609±114
Female	347±81	535±93	446±106
Average	323±59	474±60	535±79

[a]Mean ± standard error, n = 5 to 8.

90 to 180 days was larger than the increase during the third trimester of gestation. The data in Table 10 suggest a sex difference in pituitary LH, but it was not statistically significant; females had 29% more pituitary LH than males at 180 days. The LH content in the female fetal pituitaries decreased 15% between 180 and 260 days while the male LH levels increased nearly 50% during the same period, but the age-sex interaction was not significant. At 260 days the LH content in males was 36% higher than that in females.

Smith and Dortzbach (1929) reported gonadotrophic activity in fetal pig pituitaries in 16- to 17-cm C-R fetuses, but the gonadotrophic activity was much greater in 26- to 28-cm C-R pig fetuses. This observation was confirmed by Melampy et al. (1966) who measured LH by ovarian ascorbic acid depletion (OAAD) of fetal pig pituitaries; they detected LH at 80 days and markedly increased levels at 100 days. Perhaps the more sensitive RIA could be used to quantify the pituitary LH in pig fetuses younger than 80 days. Human fetal pituitary LH activity at 18 weeks was reported by Levina (1968) and at 24 weeks by Rice et al. (1968), but neither reported concentrations.

Dubois and Mauleon (1969) identified LH by immunofluorescent techniques in the sheep fetus as early as 49 days of gestation. LH was detected in the sheep fetal pituitary, both male and female, at 60 days by OAAD (Mauleon

and Reviers, 1969). Increasing LH concentrations were found as the gestation lengthened and fetal males were found to have higher pituitary LH concentrations than females near birth, both observations in agreement with the data in Table 10.

Bates et al. (1935) used bovine fetal pituitary extracts to stimulate testicular weight increases in doves. Bates indicated that FSH caused the increased testicular weight; however, his extract probably included LH activity. Karg (1967b) measured fetal bovine LH by OAAD and reported the results in terms of C-R lengths. In terms of gestation age, the values for 90, 180 and 260 days of gestation were approximately 4.8, 10.1, and 9.6 μg/mg of dried pituitary, respectively. Similar trends were obtained in this study as shown in Table 10.

Foster (1971) used RIA to quantify pituitary LH in the fetal lamb. The pituitary LH increases were greater from 55 to 139 days in the fetal lamb than in the comparable age-period in the fetal bovine (Table 10). Foster reported that male pituitary LH levels increased from 10 to 1323 ng/mg and females from 25 to 365 ng/mg during this part of the gestation period. Higher pituitary LH in male sheep fetuses (Foster, 1971) agrees with the data for cows (Table 10) near birth. Macmillan and Hafs (1968) reported 760 ng of LH (OAAD)/mg pituitary in Holstein bulls at birth; in good agreement with the data in Table 10.

I conclude that: (1) bovine fetal pituitary LH concentration increases ($P < 0.01$) with advancing fetal age, and (2) sex of the fetus appears to influence fetal pituitary LH, because the male fetus has higher pituitary LH concentrations than the female during the last trimester of gestation.

b. Growth Hormone (GH).--The bovine fetal pituitary contains considerably more GH (Table 11) than LH or prolactin (Tables 10, 12). Fetal pituitary GH levels were

TABLE 11.--Fetal anterior pituitary growth hormone content.

Sex	Days Gestation		
	90	180	260
	----------(μg/mg)----------		
Male	4.1±1.5[a]	8.9±1.9	12.1±4.3
Female	4.2±0.8	9.0±1.2	25.3±6.6
Average	4.2±0.7	8.9±1.1	18.1±4.2

[a]Mean ± standard error, n = 5 to 8.

TABLE 12.--Fetal anterior pituitary prolactin content.

Sex	Days Gestation		
	90	180	260
	----------(ng/mg)----------		
Male	85±41[a]	903±510	2593±615
Female	63±32	1397±318	2408±325
Average	72±24	1150±295	2508±351

[a]Mean ± standard error, n = 5 to 8.

4.2, 8.9, and 18.1 μg/mg for 90, 180 and 260 days of gestation, respectively. Each increase with advancing fetal age was highly significant ($P < 0.01$) and marked sex difference was apparent in the 260-day fetuses. Male bovine fetuses had only 50% as much pituitary GH as female fetuses ($P < 0.01$). The 260-day female fetal pituitary contained 2.5% GH by weight.

Pavlova et al. (1968) found GH activity in the human fetal pituitary at 9 weeks of age using a hemagglutination inhibition test. Biological activity of human fetal pituitary extracts was reported by Rice et al. (1968) in 24-week fetuses. Growth hormone also increased with fetal age in rat pituitaries. Birge et al. (1967) using the RIA technique found GH in 19-day fetal rat pituitaries and this level increased over 10-fold by day 21.

The fetal pig pituitary contained GH activity by 9 cm C-R while LH activity was not present until the pig fetus was 16 to 17 cm C-R (Smith and Dortzbach, 1929). Acidophils containing GH were identified in fetal sheep pituitaries after 43 days of gestation (Stokes and Boda, 1968). They found GH-containing acidophils increased as gestation lengthened; they predominated in 88-day and older sheep fetuses.

In a study of 10- to 55-cm C-R bovine fetal pituitaries using immunofluorescent techniques, Meneghelli and Scapinelli (1962) found GH in pituitaries from 18-cm fetuses.

Numbers of fetal pituitary acidophils apparently increased parallel with increases in GH-containing cells in fetuses up to 55-cm C-R. Bovine fetal pituitary GH (Table 11) averaged 4.2 μg/mg in the 90-day fetuses with an average C-R length of 22.5 cm. Two fetuses averaged 19.1 cm C-R and both had radioimmunologically reactive GH. Thus, the RIA data on bovine fetal pituitary GH agree with the immunofluorescent studies of Meneghelli and Scapinelli (1962).

I conclude that (1) bovine fetal pituitary GH is present in 90-day fetuses and increases with fetal age similar to other species, and (2) higher GH in pituitaries from females at 260 days has not been reported previously. The function of higher pituitary GH in female than male fetuses is unknown.

c. Prolactin.--Bovine fetal pituitary prolactin (Table 12) increased from 72 to 2508 ng/ml as fetal age increases ($P < 0.01$) from 90 to 260 days. Levels of fetal pituitary prolactin were intermediate to LH and GH at 260 days. In contrast to LH and GH, no sexual differences were observed in fetal pituitary prolactin.

Bates et al. (1935) reported that prolactin was present in late term fetuses, and Reece and Turner (1937) found that fetal prolactin (lactogen) was much lower than lactogen levels in calves. Fetal pituitaries averaged 0.74 bird units/pituitary, while calves averaged 111.4.

Immunofluorescent localization of prolactin in 80-day fetal sheep pituitaries has been reported by Stokes and Boda (1968). They detected two types of acidophils in the anterior pituitary. The prolactin acidophils were less numerous than the GH acidophils.

The data in Table 12 represent the first study of fetal pituitary prolactin concentrations in the bovine where RIA was used.

2. In Vitro Synthesis by Pituitary Tissue

The net *in vitro* hormone synthesis per milligram of anterior pituitary was calculated by adding the hormone in the media and that in the explants after the 72-hour incubation and subtracting that in the fresh-frozen pituitaries. Two-factor (sex and age) analysis of variance was used to statistically analyze the content and net synthesis data.

a. Luteinizing Hormone (LH).--Net synthesis of LH *in vitro* (Table 13) was greater (434 vs. 213 ng/mg) by 180-day fetal pituitaries than 260-day fetal pituitaries.

TABLE 13.--Net synthesis of LH synthesis by fetal anterior pituitary slices *in vitro*.

Sex	Days Gestation	
	180	260
	------(ng/mg/72 hr)----------	
Male	655±169[a]	273±230
Female	212±144	141±153
Average	434±128	213±138

[a]Mean ± standard error, n = 5 or 6.

Male pituitaries synthesized more (P ∿ 0.18) LH *in vitro* than females. Fetal pituitaries from 180-day male fetuses synthesized more than twice as much LH as any other age or sex.

Gey *et al.* (1938) incubated human fetal pituitaries and found that when the incubation medium was injected into immature rats it produced no macroscopic changes, although microscopic examination of the ovaries suggested gonadotrophic stimulation. Recently, Gailani *et al.* (1970) identified LH with RIA in the media after incubation of human fetal pituitaries.

I conclude that bovine fetal pituitaries produce LH during *in vitro* incubation and that age and sex of the fetus appear to influence the capacity of the pituitary tissue to synthesize LH.

b. Growth Hormone (GH).--Bovine fetal pituitary synthesis of GH *in vitro* (Table 14) by female fetal pituitaries was appreciably higher than in males, however, the

TABLE 14.--Net synthesis of growth hormone by fetal anterior pituitary slices *in vitro*.

Sex	Days Gestation	
	180	260
	-------(μg/mg/72 hr)--------	
Male	1.2±2.6[a]	0.7±2.5
Female	17±19	2.8±4.8
Average	8.7±9.5	1.7±2.5

[a]Mean ± standard error, n = 5 or 6.

variations between animals within age and sex were very large. The incubation medium contained high concentrations of GH, but much of this was released by the pituitary tissue during incubation rather than from net synthesis of GH (Appendix VIII).

Gitlin and Biasucci (1969), incubating pituitaries with radioactive amino acids, reported *in vitro* synthesis of radioactive GH by human fetal pituitaries from 9-week fetuses. Gailani *et al*. (1970) showed that media from human fetal pituitary incubations stimulated rat tibial growth, suggesting the presence of GH, and RIA was used to quantify the GH synthesized. Thus, the human pituitary GH synthesized *in vitro* has both immunological and biological activity. I conclude that the bovine fetal pituitary is also capable of *in vitro* synthesis of GH.

c. Prolactin.--Fetal pituitary synthesis of prolactin *in vitro* (Table 15) averaged 24 μg/mg for the 72-hour incubation. Fetal age did not appear to effect prolactin synthesis, but the influence of sex of pituitaries from female fetuses synthesized about three times more prolactin *in vitro* than males ($P \sim 0.07$).

Brauman *et al*. (1964) reported that *in vitro* synthesis of prolactin by human fetal pituitaries increased with increasing time of incubation up to 33 days, but net synthesis of GH decreased.

TABLE 15.--Net synthesis of prolactin by fetal anterior pituitary slices *in vitro*.

Sex	Days Gestation 180	260
	----(μg/mg/72 hr)---	
Male	10.4±3.0[a]	12.1±3.5
Female	37.5±24.0	36.5±10.0
Average	24.0±12.5	23.2±6.2

[a]Mean ± standard error, n = 5 or 6.

I conclude that bovine fetal pituitaries synthesize prolactin *in vitro* and that female pituitaries probably synthesize more prolactin than pituitaries from male fetuses.

3. Hormones in Fetal Blood Serum

The data on peripheral hormones were analyzed statistically in two steps. The first step involved a two-factor (sex and age) analysis of variance for the 90, 180 and 260-day fetuses. Second the 180-day and 260-day fetal data were analyzed by using three-factor (fetal sex, age and source of blood) analysis of variance. The two step procedure was necessary because data were not available on arterial and venous hormone levels for 90-day fetuses.

a. Luteinizing Hormone (LH).--Fetal serum LH (Table 16) for 90-day fetuses averaged 3.0 ng/ml and decreased to 1.28 ng/ml at 180 days and to 0.85 ng/ml at 260 days ($P < 0.01$). Females averaged higher serum LH than males at 90 and at 180 days ($P < 0.01$). The 90-day female

TABLE 16.--Average fetal serum LH.[a]

Sex	Days Gestation 90	180	260
	----------(ng/ml)----------		
Male	1.46±0.15[b]	1.14±0.23	0.88±0.12
Female	3.9±0.60	1.47±0.19	0.83±0.18
Average	3.0±0.5	1.28±0.15	0.85±0.10

[a]180 and 260 day values represent the average of umbilical artery and vein.

[b]Mean ± standard error, n = 5 to 8.

fetuses (3.9 ng/ml) averaging about 170% higher than 90-day males. The 260-day males, however, had higher LH than the females, causing a sex-age interaction ($P < 0.01$).

Foster (1971) reported serum LH levels in fetal sheep of comparable ages. Serum LH for 55-, 100- and 139-day sheep fetuses were 0.25, 1.0 and 0.30 ng/ml for single male fetuses and 0.70, 1.1 and 0.20 ng/ml for single female fetuses, respectively. Thus, the sex differences in serum LH levels for each age group are qualitatively similar for sheep and bovine fetuses. However, the sheep fetal serum LH appeared to peak near the end of the second trimester of gestation while the highest serum LH for the bovine fetuses was found in the first trimester.

I found no significant difference in fetal arterial and venous serum LH (Table 17). These results suggest that placental transfer, production or uptake of LH is minimal, and the circulating LH serum levels remain relatively constant.

TABLE 17.--Average fetal serum LH in the umbilical artery and vein.

Sex	Source	Days Gestation 180	260
		----------(ng/ml)----------	
Male	Artery	1.12±0.35[a]	0.87±0.18
	Vein	1.14±0.34	0.88±0.17
Female	Artery	1.47±0.29	0.84±0.26
	Vein	1.47±0.26	0.82±0.27
Average	Artery	1.28±0.23	0.85±0.14
	Vein	1.29±0.22	0.85±0.15

[a]Mean ± standard error, n = 5 to 7.

A comparison of fetal pituitary LH to serum LH (Figure 6) revealed that pituitary LH increased with fetal age while the serum LH declined. Therefore, fetal pituitary LH was correlated ($r = 0.41$, $P < 0.05$) with the C-R lengths while the serum LH was inversely related to C-R length ($r = -0.58$, $P < 0.05$).

b. Growth Hormone (GH).--Growth hormone in fetal serum increased 140% ($P < 0.01$) from 90 days to 260 days. Serum GH was 42 ng/ml for 90-day fetuses and increased to 65 and 103 ng/ml for 180- and 260-day fetuses, respectively (Table 18). Although the 90-day female fetuses averaged 70% more serum GH than comparable males, the sex difference was not significant over all three stages of gestation.

Differences in fetal arterial and venous GH levels were insignificant, and only fetal age differences were apparent ($P < 0.01$, Table 19). Bassett et al. (1970)

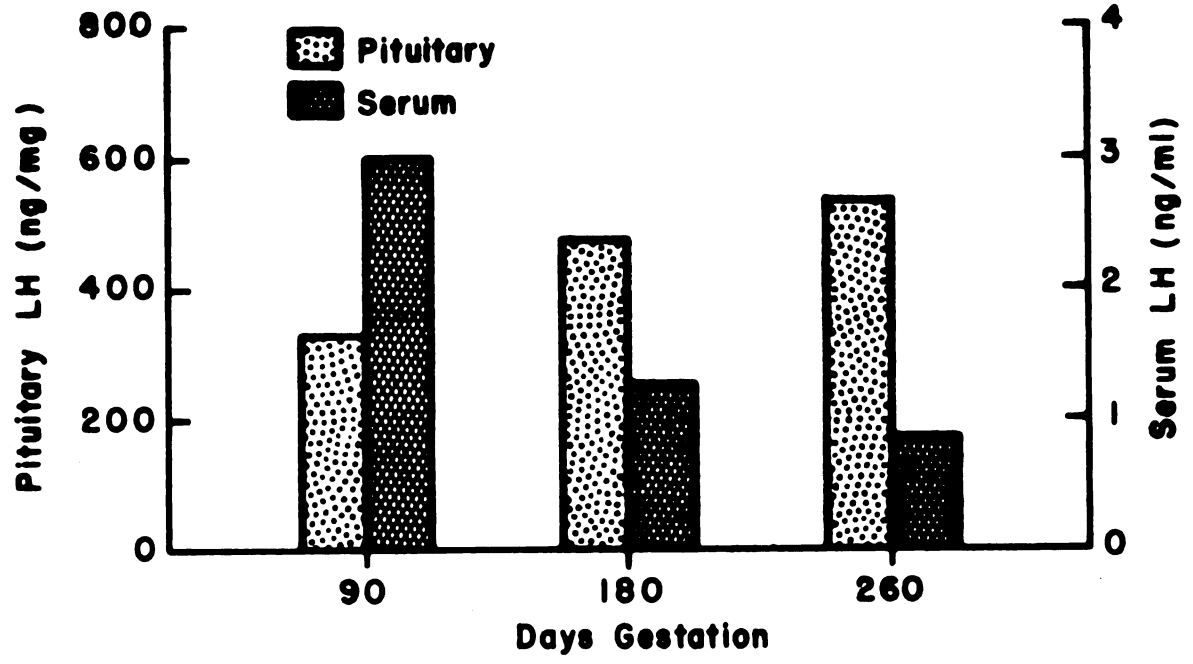


Figure 6 Levels of LH in fetal pituitary and blood serum.

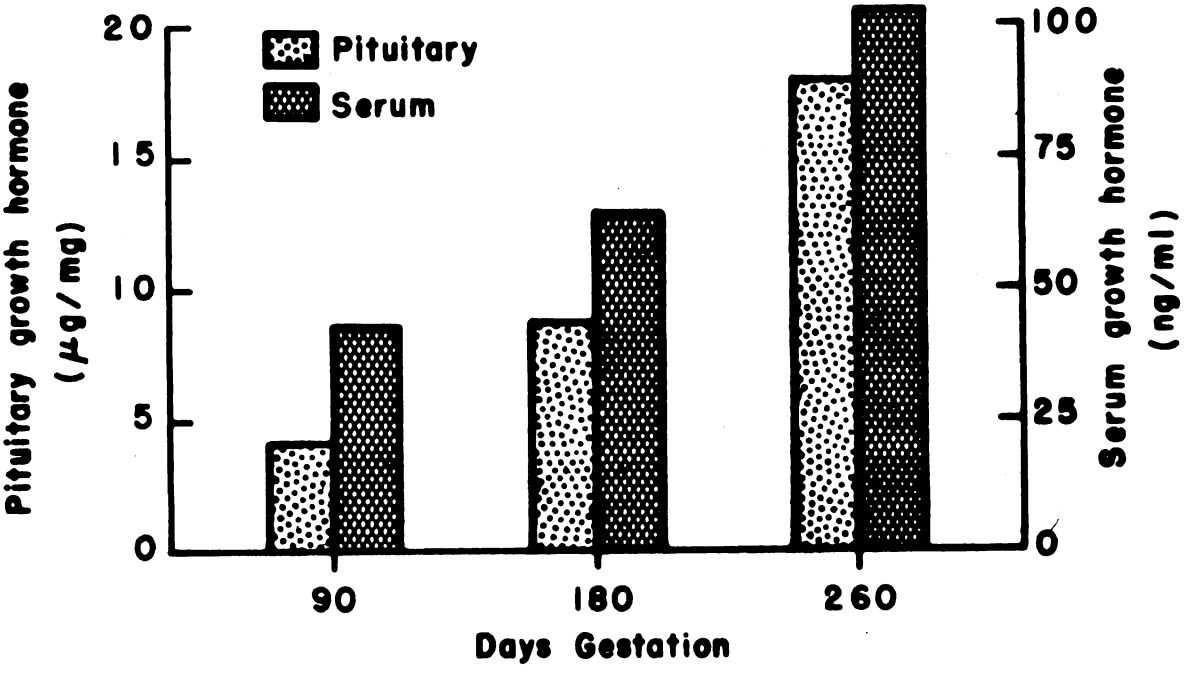


Figure 7 Levels of growth hormone in fetal pituitary and blood serum.

TABLE 18.--Average fetal serum growth hormone.[a]

Sex	Days Gestation 90	180	260
	----------(ng/ml)----------		
Male	29±4[b]	64±7	109±8
Female	50±12	65±7	97±15
Average	42±8	65±5	103±8

[a]180 and 260 day values represent the average of the umbilical artery and vein.

[b]Mean ± standard error, n = 5 to 8.

TABLE 19.--Average fetal serum growth hormone in the umbilical artery and vein.

Sex	Source	Days Gestation 180	260
		--------(ng/ml)----------	
Male	Artery	65±10[a]	109±11
	Vein	64±11	109±13
Female	Artery	66±9	93±20
	Vein	65±12	100±26
Average	Artery	65±7	102±10
	Vein	64±8	105±13

[a]Mean ± standard error, n = 5 to 7.

reported serum GH levels of 40 to 50 ng/ml and 110 to 120 ng/ml for 110 and 135-day fetal sheep, respectively.

Bassett et al. (1970) also found that serum GH levels decreased rapidly after fetal hypophysectomy; exogenous and endogenous GH in the serum decreased at similar rates. Furthermore infusion of Isoprenaline (isopropyl

nonadrenaline hydrochloride, Sigma) depressed fetal sheep serum GH levels, but GH quickly returned to normal after cessation of isoprenaline infusion. Thus fetal serum GH levels apparently are controlled by the fetus.

Both fetal pituitary and serum concentrations of GH increase with fetal age ($r = 0.41$, $P < 0.01$, Figure 7). The correlations of C-R length with pituitary GH levels ($r = 0.60$) and of C-R length with serum GH levels ($r = 0.57$) were significant. ($P < 0.01$). Therefore the observed increases in serum GH levels do not arise from decreased fetal pituitary GH. The physiological significance of GH in the fetus has not been determined, although the high GH levels occur during the period of most rapid relative growth of the fetus.

c. Prolactin.--Fetal serum prolactin was low in 90-day fetuses (3.6 ng/ml) but increased over 10-fold at 180 days of gestation (43 ng/ml) and rose to 61 mg/ml at 260 days (Table 20). These prolactin changes ($P < 0.01$)

TABLE 20.--Average fetal serum prolactin levels.[a]

Sex	Days Gestation		
	90	180	260
	(ng/ml)		
Male	3.5±0.4[b]	30±5	60±17
Female	3.7±0.6	58±12	63±11
Average	3.6±0.4	43±7	61±10

[a]180 and 260 day values represent the average of umbilical artery and vein.

[b]Mean ± standard error, n = 5 to 8.

resemble those for GH, and the fetal serum prolactin and GH were correlated ($r = 0.45$, $P < 0.01$). Sex differences in serum prolactin levels were not detected, but the interaction ($P \sim 0.09$) of sex and age was observed. This relationship was produced primarily by a 50% lower prolactin in serum from 180-day male fetuses as compared to the females.

Serum prolactin in 180- and 260-day fetuses was higher in umbilical arterial blood (Table 21) than in venous blood ($P \sim 0.20$), suggesting placental uptake or transfer but not production of fetal prolactin.

TABLE 21.--Average fetal serum prolactin in the umbilical artery and vein.

Sex	Source	Days Gestation 180	260
		--------(ng/ml)---------	
Male	Artery	34±8[a]	59±22
	Vein	26±6	61±27
Female	Artery	58±20	68±19
	Vein	57±16	57±14
Average	Artery	45±10	63±14
	Vein	40±9	59±15

[a]Mean ± standard error, n = 5 to 7.

When fetal pituitary and serum prolactin concentrations are compared ($r = 0.62$, Figure 8), a parallel increase was evident during gestation. The pituitary prolactin ($r = 0.78$) and the serum prolactin ($r = 0.57$) levels appear to increase with fetal age. At the present

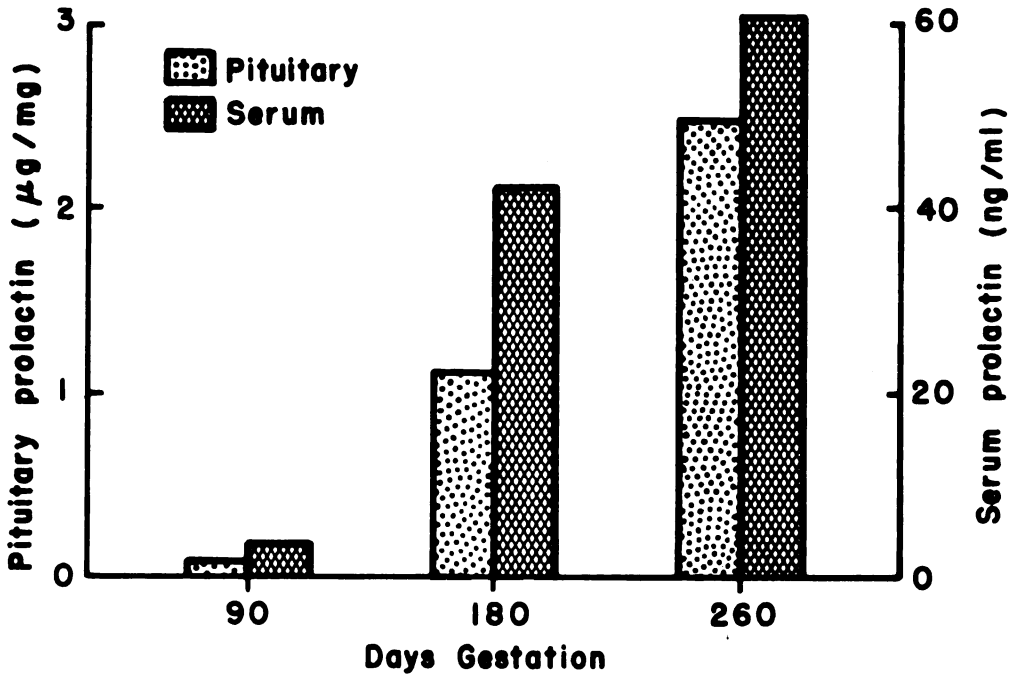


Figure 8 Levels of prolactin in fetal pituitary and serum.

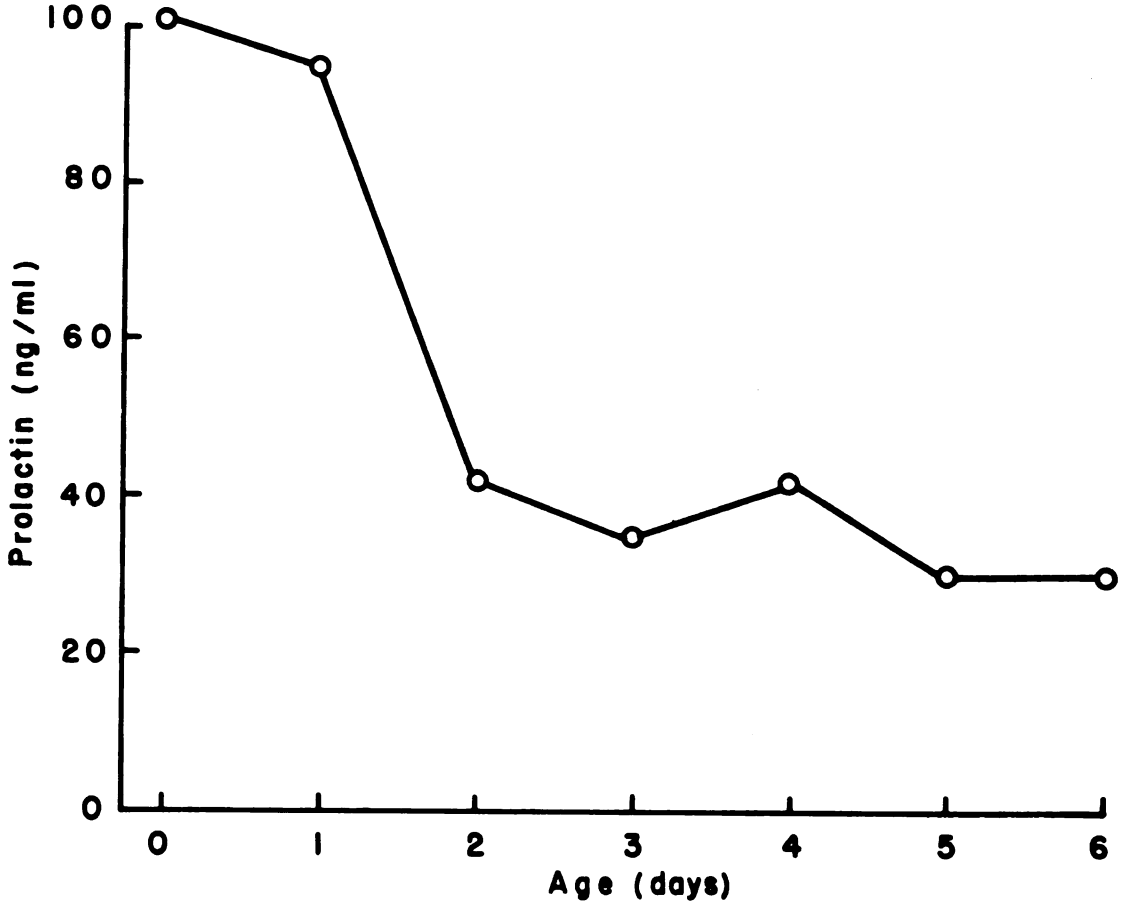


Figure 9 Neonatal calf jugular serum prolactin.

time the significance of the prolactin in the fetus also remains obscure, but the A-V difference across the placenta suggests fetal prolactin may aid placental function.

D. Neonatal Serum Hormones

The serum hormone data for neonates was treated statistically by analysis of variance with sex and age as variables. Age-changes were compared by orthogonal contrast.

1. Luteinizing Hormone (LH)

Since sex differences for serum LH levels were not observed, the values for males and females were combined in Table 22. The average serum LH level ranges from 0.36 to 0.49 ng/ml for calves from birth to 6 days of age; there were no significant changes in serum LH during the first 6 days.

TABLE 22.--Neonatal jugular serum growth hormone, LH and prolactin.

Age	Growth Hormone	LH	Prolactin
(days)	----------	(ng/ml)	----------
0	36±9	0.36±0.11	101±24
1	28±4	0.49±0.06	95±25
2	28±8	0.46±0.05	42±8
3	24±4	0.46±0.06	34±10
4	18±3	0.48±0.08	43±10
5	24±6	0.49±0.06	30±5
6	32±5	0.40±0.06	30±6

[a]Mean ± standard error, n = 9 or 10.

Macmillan and Hafs (1968) reported that plasma LH levels averaged 0.48 ng/ml in bulls between 1 and 7 days old. The serum LH in lambs 1 to 6 days old averaged from 0.20 to 0.60 ng/ml (Foster, 1971). Values of neonatal serum LH in other species were not available.

2. Growth Hormone (GH)

Serum GH levels for male and female calves were similar, therefore the data were combined in Table 22. Daily averages ranged from 18 to 36 ng/ml of serum but no significant variation with age was detected.

The growth hormone levels shown in Table 22 agree with previously reported data by Purchas et al. (1970). Grumbach et al. (1968) reported that human fetal umbilical cord blood contained 33.5 ng/ml of GH.

3. Prolactin

Daily average serum prolactin levels are reported in Table 22 for calves from birth to 6 days of age. The values for males and females were again combined since no sex difference was observed. A significant and rapid decrease in serum prolactin level occurred between day 1 and day 2 ($P < 0.01$). This decrease is shown graphically in Figure 9 (p. 88). Both the day of birth and day of birth plus day 1 were significantly higher ($P < 0.01$) than the averages for the remaining days. Other reports on neonatal prolactin levels were unavailable.

GENERAL DISCUSSION

A. Maternal-Fetal Hormone Interactions

1. Growth Hormone (GH)

When the average GH in serum from the three blood sources taken from cows was compared with the average GH in fetal serum (Figure 10), fetal GH averaged 10 to 20 times higher than maternal GH ($P < 0.01$). Human fetal serum GH has been found to be six times higher than maternal levels at birth (Hutchinson et al., 1962). While Bassett et al. (1970) only compared the fetal sheep-maternal serum GH during the third trimester, they also reported fetal levels ten times higher than maternal levels.

In at least the cow, sheep and human, fetal serum GH levels are much higher than for any period after birth. The physiological significance of high fetal serum GH remains obscure since fetal growth appears to proceed almost normally in hypophysectomized lamb fetuses (Liggins and Kennedy, 1968).

Placental transfer of GH appears minimal, because a large concentration gradient was maintained across the placenta throughout pregnancy. Gitlin et al. (1965) injected radioactive GH into pregnant women and could not detect placental transfer of labeled GH to the fetus. If

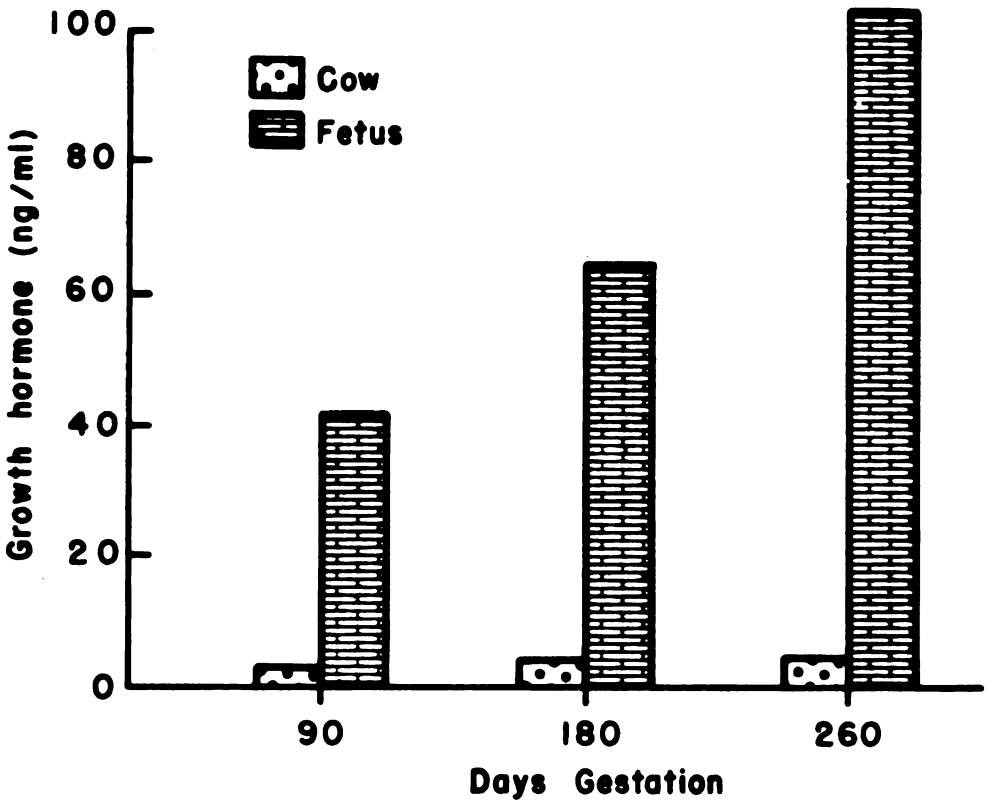


Figure 10 Levels of growth hormone in cow and fetal blood sera during gestation.

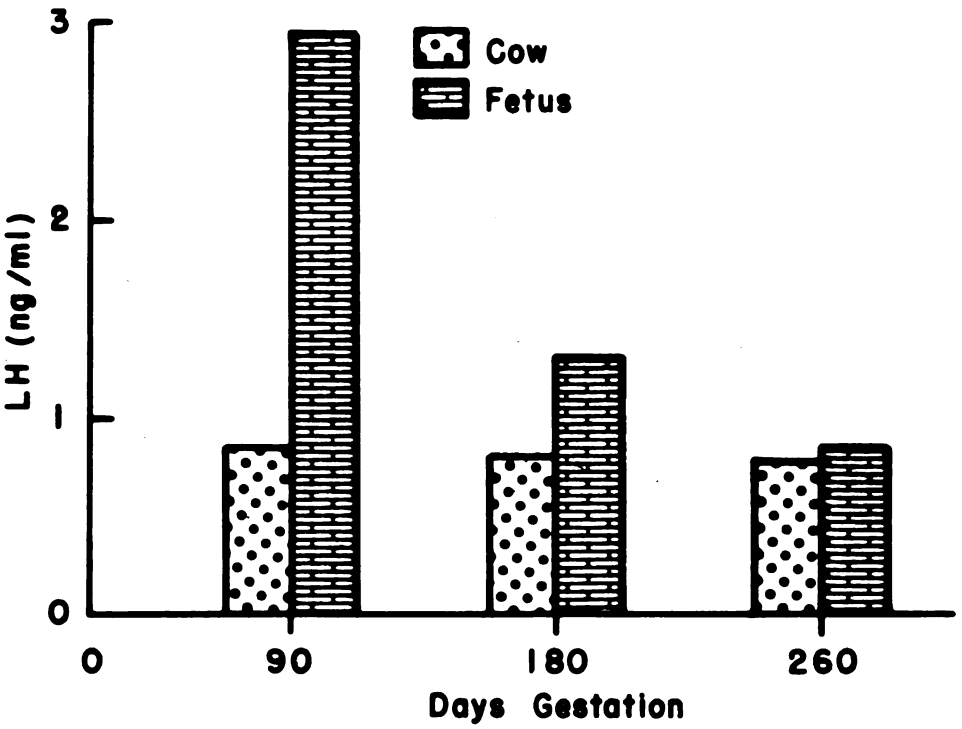


Figure 11 Levels of LH in cow and fetal blood sera during gestation.

the bovine placenta is equally impermeable to GH as the data in Figure 10 suggest, then the high circulating fetal levels must arise from the fetal pituitary. As further evidence to support this hypothesis, Bassett et al. (1970) observed that sheep fetal GH levels rapidly decreased after fetal hypophysectomy. That I found no AV differences in GH across the placenta also supports the suggestion that GH is neither taken up, produced nor transferred by the placenta.

2. Luteinizing Hormone (LH)

A comparison of the maternal serum LH (average of jugular vein, uterine artery and uterine vein) to the average in fetal serum LH is shown in Figure 11. The 90-day fetal serum LH is three times higher than maternal serum LH, but this difference in serum LH levels decreased by 180 days and nearly disappeared at 260 days of gestation. As was found for GH, the placenta maintains serum LH concentration differences between the fetal and maternal systems.

Foster (1971) injected exogenous LH to raise the level of either the fetal or the maternal serum LH of sheep and showed that the sheep placenta does not transfer LH in either direction. If the same is true in the cow, as the data in Figure 11 suggest, then bovine fetal serum LH must arise from the fetal pituitary. Absence of AV differences in LH across the placenta also supports this suggestion.

3. Prolactin

Serum prolactin in the cow (average of jugular vein, uterine artery and uterine vein) is compared with average fetal serum prolactin levels in Figure 12. In contrast to the results for LH and GH, fetal serum prolactin levels were lower than the maternal levels during all three trimesters of gestation, and maternal serum prolactin averaged from 3 to 30 times higher than fetal serum levels. But as for GH and LH, placental transfer of prolactin appeared to be minimal.

Three different patterns are observed for these three hormones. Fetal GH and LH levels were higher than maternal levels during gestation, but GH increased while the LH decreased. Serum prolactin which was higher in maternal serum than in fetal serum increased during gestation.

The above variations in the ratios of the fetal to the maternal serum levels of GH, LH and prolactin all support the hypothesis that the bovine placenta does not transfer, take up, or produce these hormones.

4. Effect of Fetal Sex

Another interesting fetal-maternal relationship in this study was the effect of the sex of the fetus on maternal GH and the prolactin (Table 6). Cows carrying male fetuses averaged nearly twice as high GH levels in the jugular vein as cows with female fetuses at both 180

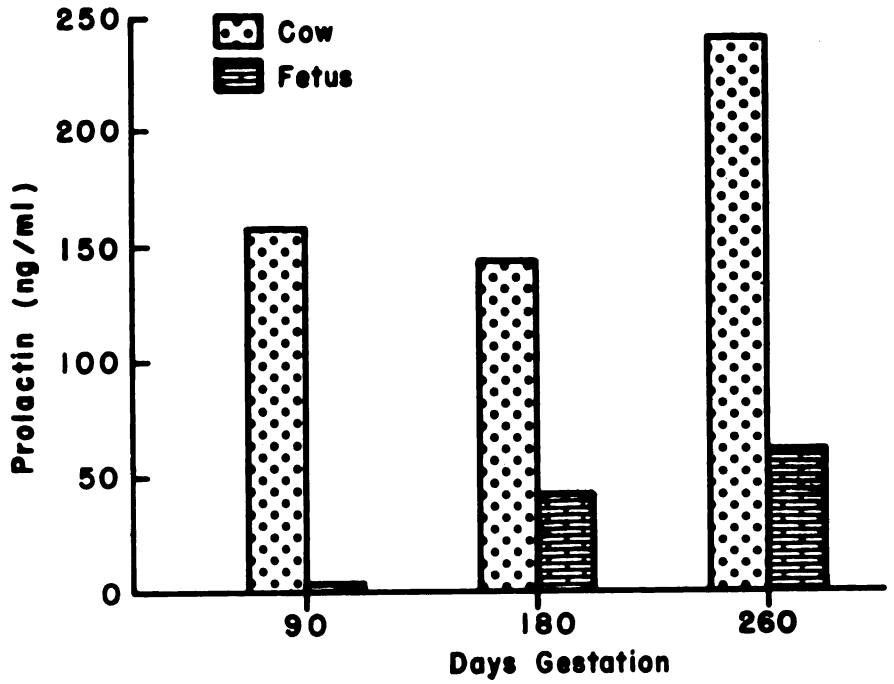


Figure 12 Levels of prolactin in cow and fetal blood sera during gestation.

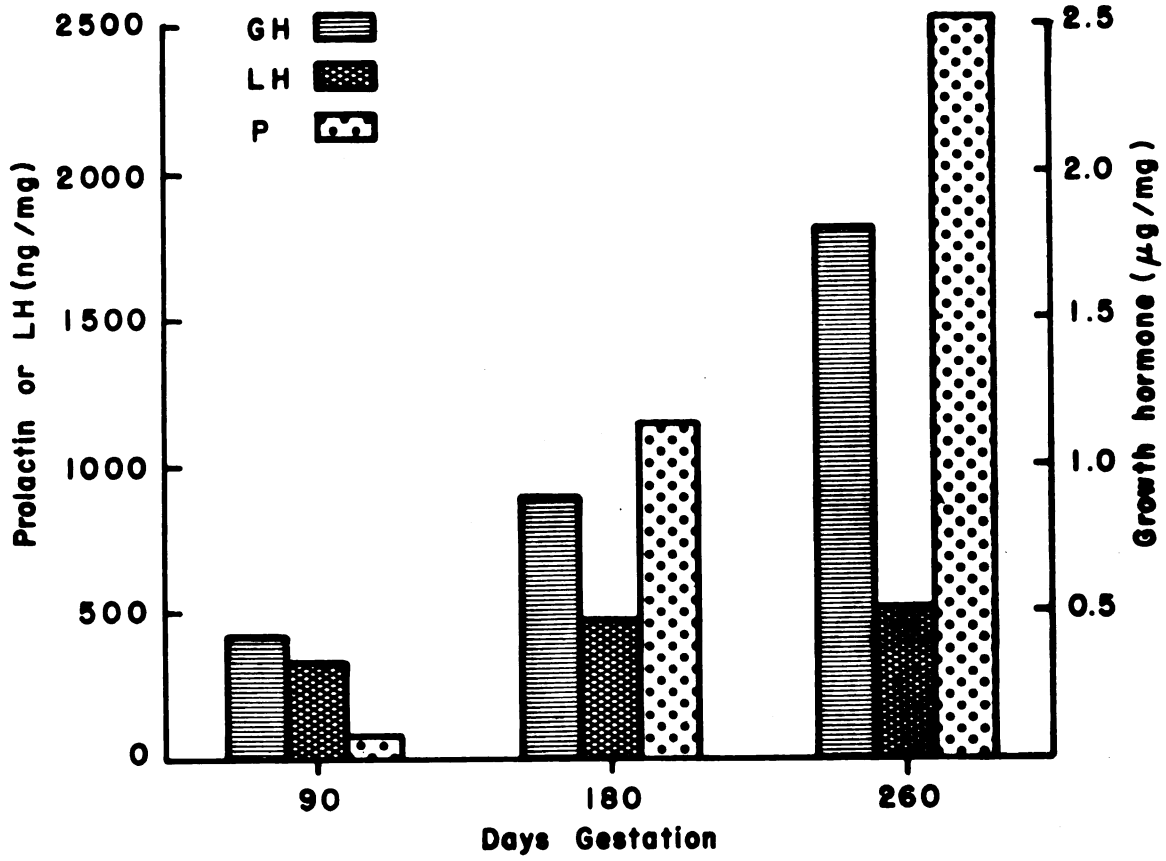


Figure 13 Fetal anterior pituitary growth hormone, LH and prolactin.

and 260 days of gestation ($P \sim 0.11$). The difference in maternal serum prolactin levels due to the sex of the fetus (Table 6) was greater for prolactin ($P < 0.05$) than for GH. Furthermore, it was evident early in gestation (90 days), because cows with male fetuses averaged more than three times higher serum prolactin levels than cows with female fetuses at 90 days of gestation. At 260 days of gestation cows carrying male fetuses were more than six times higher.

Sex of the developing fetus previously was reported to alter maternal endocrine and metabolic functions. Primates carrying male fetuses had higher blood testosterone levels (Resko, 1970). Women carrying male babies averaged higher HCG levels (Brody and Carlström, 1965) and also excreted more pregnanediol (Rawlings and Krieger, 1964).

A possible mechanism for fetal control of maternal hormone levels may be androgen transfer from the fetal to the maternal system. The fetal androgen then may act on the maternal hypothalamus or pituitary.

An important aspect of maternal-fetal relationships has been reported by MacMillan (1970). He found that women who delivered abnormally small babies had significantly lower serum human chorionic somatomammotropin levels. An understanding of the basis for these observations is desirable to prevent abnormal developments.

Shane et al. (1969) caused pseudohermaphroditism in female dog fetuses when the pregnant dog was fed methyl testosterone. Coxofemoral hypoplasia and premature epiphyseal ossification was reported by Gustafsson and Beling (1969) in pups from mothers that received estradiol during gestation. Both of these reports demonstrated maternal transfer of steroid hormones to the fetus. Medical implications of developmental defects due to maternal medication are obvious. Clinical problems such as coxofemoral hypoplasia are not uncommon.

B. Fetal Pituitary and Serum Hormone Comparisons

1. Pituitary Content

Comparisons of fetal pituitary hormone levels at all three trimesters of gestation are shown in Figure 13. The pituitary concentration of GH, LH and prolactin all increased with fetal age. GH was approximately ten times higher than LH or prolactin.

GH apparently is one of the first hormones that can be identified in quantity in the fetal pituitary. Gitlin and Biasucci (1969) reported 60-day human fetal pituitaries can synthesize GH. Acidophils containing GH were found in fetal pituitaries from the 43-day sheep (Stokes and Boda, 1968) and the 18-cm C-R bovine (Meneghelli and Scapinelli, 1962). These GH cells increased in number as the fetal age advanced. Because GH cells in the pituitary were most numerous early in fetal life, it was not surprising that GH

levels in the 90-day pituitary were higher than for LH and prolactin (Figure 13).

Fetal pituitary LH increased from 90 to 180 days ($P < 0.01$), but little change was evident between 180 and 260 days. The LH level was approximately five times higher than the prolactin level in the 90-day fetal pituitary. However, prolactin levels were two to four times higher than LH levels in 180- and 260-day fetal pituitaries. Cells containing LH have been identified in the 49-day fetal sheep pituitary (Dubois and Mauleon, 1969). Biological LH activity has also been reported by Mauleon and Reviers (1969) in 60-day fetal sheep pituitaries. Foster (1971), using RIA, quantified LH in 55-day fetal sheep pituitaries and demonstrated the biological activity of fetal LH. The above reports on fetal sheep pituitary LH correspond to fetal ages approximately equal to the 90-day bovine fetus. The pituitary LH concentrations was more than 300 ng/mg at 90 days in the bovine fetus. How soon after conception LH may be detected by RIA remains to be tested.

Although Reece and Turner (1937) found prolactin in bovine fetal pituitaries, they only studied fetuses in late gestation. Stokes and Boda(1968) found prolactin containing cells in 80-day sheep fetuses. Measureable (RIA) quantities of prolactin were also present in the 90-day bovine fetal pituitaries (Figure 13). Of the three hormones studied (GH, LH and prolactin), prolactin may be the last to appear in the bovine fetal pituitary.

2. In Vitro Synthesis

Comparative synthesis of GH, LH and prolactin by anterior pituitary slices *in vitro* is shown in Figure 14. A significant quantity of GH, LH and prolactin was synthesized *in vitro*. The correlation between pituitary GH content and *in vitro* synthesis ($r = -0.09$) was not significant. The only correlation that was significant ($P < 0.05$) between GH content and *in vitro* synthesis was the 180-day male fetal pituitaries ($r = -0.78$, Table 23). Biological and immunological (RIA) activity of GH produced by incubation of human fetal pituitary tissues was reported by Gailani, *et al*. (1970).

TABLE 23.--Some correlations between fetal pituitary hormone concentrations and net *in vitro* hormone synthesis.

Days of gestation	Sex	Pituitary vs. net synthesis in vitro		
		GH	LH	Prolactin
180	Male	-0.78[a]	0.17	0.55
	Female	0.41	0.15	0.48
260	Male	0.01	0.00	-0.28
	Female	-0.42	-0.70	0.89[a]

[a] $P < 0.05$.

In vitro synthesis of LH also is compared with the pituitary LH content in Figure 14. Correlations, within sex and age, of LH synthesis compared with pituitary LH content are shown in Table 23 and were not statistically significant. Previous studies with tissue cultures of human

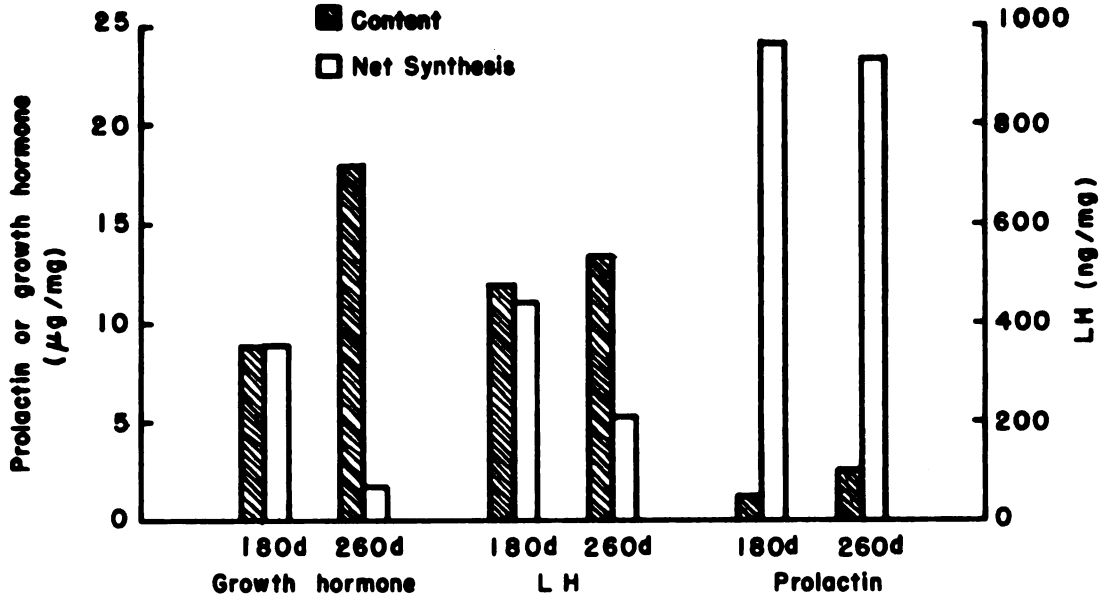


Figure 14 Fetal pituitary content and in vitro synthesis of growth hormone, LH and prolactin.

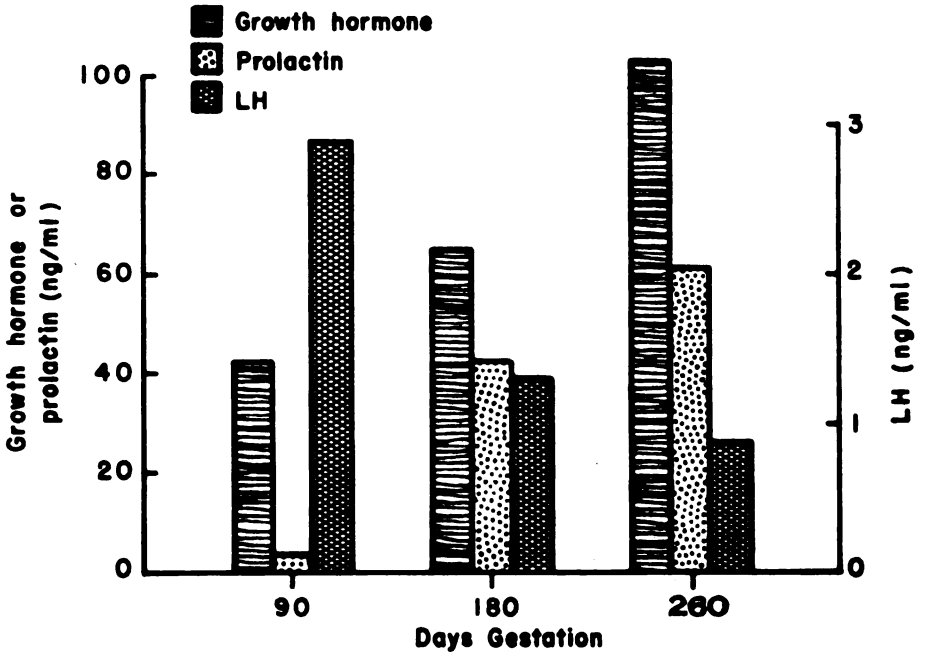


Figure 15 Fetal blood serum growth hormone, LH and prolactin during gestation.

fetal pituitary by Gey *et al*. (1938) indicated that LH may be synthesized *in vitro*. Also, Gitlin and Biasucci (1969) and Gailani *et al*. (1970) showed that LH was synthesized in human fetal tissue cultures.

In vitro prolactin synthesis by fetal pituitary tissue (Figure 14) is much greater than the net synthesis of either LH or GH. Female pituitaries synthesized more prolactin during incubation than pituitaries from males ($P \sim 0.06$, Table 15). The correlations within sex and age groups are shown in Table 23. The 260-day female fetal pituitary content was correlated to *in vitro* prolactin production ($r = 0.89$, $P < 0.05$). Brauman *et al*. (1964) reported increasing synthesis of prolactin by fetal pituitary cells up to 33 days of incubation.

Fetal sex and age appear to influence the synthetic capacity of pituitary tissue. The relatively low (Table 14, Appendix VIII) net synthesis of GH during incubation may have resulted from feed-back inhibition, because the medium levels of GH became very high. Another explanation for low net synthesis of GH and LH compared with prolactin may be the requirement for hypothalamic stimulation for the synthesis of GH and LH. Generally, hypothalamic action on pituitary GH and LH is stimulatory, while it inhibits prolactin synthesis and release. *In vitro* prolactin synthesis appears to be enhanced because hypothamic inhibition has been removed. Because *in vitro* synthesis of hormones

in fetal pituitaries differed in males and females, the fetal gonads apparently influenced pituitary function before 180 days. At 180 days the female pituitaries synthesized 3-fold more prolactin than pituitaries from males.

3. Serum Levels

Fetal serum LH decreases (Figure 15) with increasing fetal age ($P < 0.05$), while both GH and prolactin serum levels increase as the gestation period lengthens ($P < 0.01$, $P \sim 0.08$, respectively). These differential age-related changes indicate a fetal control system functions during development. In support of this hypothesis, sex of fetus caused significant ($P < 0.01$) differences in fetal serum LH. Foster (1971) reported similar sex and age differences in fetal sheep serum LH levels.

Foster injected exogenous LH releasing factor to determine if the fetal pituitary would respond by releasing LH. Although the variation in response to LH releasing factor was large, evidence of LH release was reported.

Table 24 lists coefficients of correlation between fetal pituitary hormone concentrations and fetal serum hormones within sex and age groups. Overall, pituitary and the serum levels of GH ($r = 0.41$) and prolactin ($r = 0.62$) were significantly correlated ($P < 0.01$). But within age and sex groups, only those between GH in the pituitary and serum of the 90-day males ($r = 0.79$, $P < 0.05$) and between pituitary and serum prolactin for 180-day males

TABLE 24.--Some correlations between fetal pituitary and fetal serum[a] concentrations of hormones.

Days of gestation	Sex	Pituitary vs. Serum Concentration		
		GH	LH	Prolactin
90	Male	0.79[b]	0.08	0.14
	Female	0.11	0.59	0.26
180	Male	-0.11	0.21	0.98[c]
	Female	0.33	0.40	0.72
260	Male	-0.69	0.29	-0.05
	Female	0.57	-0.32	0.50

[a]Average of umbilical artery and umbilical vein.

[b]$P < 0.05$.

[c]$P < 0.01$.

were significant ($r = 0.98$, $P < 0.01$, Table 24). These latter two groups are small numbers ($N = 5$) and caution should be used in interpreting the results. The overall correlations are probably meaningless, since they are composed of significantly different components.

Correlations between pituitary synthesis in vitro and fetal serum hormone levels are listed in Table 25. Overall, none of the correlations between in vitro and fetal serum hormone levels was significant. However, pituitary synthesis and serum levels were significantly correlated within certain sex and age groups. Differences among correlations for the three hormones within age and sex groups may indicate fetal pituitary hormone synthesis and release are controlled differently for the three hormones.

TABLE 25.--Some correlations between fetal serum[a] hormones and pituitary net *in vitro* synthesis.

Days of gestation	Sex	Serum hormone levels vs. net synthesis in vitro		
		GH	LH	Prolactin
180	Male	0.08	-0.71	0.65
	Female	0.97[c]	-0.02	0.46
260	Male	0.39	0.91[c]	-0.31
	Female	0.10	0.71	0.80[b]

[a]Average of umbilical artery and umbilical vein.

[b]$P < 0.05$.

[c]$P < 0.01$.

To my knowledge, all hormones found in adults also have been found in the fetuses of one or more species of animals. Whether the hormones function in the developing fetus in the same manner as the adult has not been established. A few functions of fetal hormones, however, appear well documented and are reviewed by Jost and Picon (1970). Fetal testicular stimulation by fetal pituitary gonadotropin (LH) during development apparently occurs in most species, and liver glycogen synthesis in the fetus appears to require fetal GH. No function requiring fetal prolactin has been reported. Although I did not study fetal adrenal hormones, others showed that fetal pituitary ACTH controls fetal serum adrenal corticoid levels (Kitchell and Wells, 1952) and that fetal corticoids participate in the initiation of parturition (Drost and Holm, 1968).

C. Fetal-Neonatal Serum Hormones

Fetal endocrine development is a gradual process that continues up to birth (Figure 16). LH was highest at 90 days and gradually decreased until birth. The LH level at birth did not change significantly during the first 6 days of life in the calf. These data agree well with the serum LH levels Foster (1971) reported for fetal and neonatal sheep.

Serum prolactin levels gradually rose from 90 days to the highest level at birth (>100 ng/ml, Figure 16), and then decreased sharply to 30-40 ng/ml by day 2 of life. More frequent antenatal sampling would be necessary to determine if the serum prolactin increases as rapidly prior to birth as the postnatal decrease. No function of the relatively high prolactin levels at birth has been determined. High serum prolactin levels may be caused by stresses to the calf during parturition, since Tucker (1971) and Raud et al. (1971) showed that stress can cause release of prolactin in cows.

Fetal serum GH increased from 40 ng/ml at 90 days to a peak of 100 ng/ml at 260 days. But, at birth, GH dropped to an average of 36 ng/ml and remained relatively constant during the first week following birth. Since intermediate samples between 260 days and birth were not taken, when GH dropped during this time is not known. The sharp changes in GH and prolactin near birth while LH

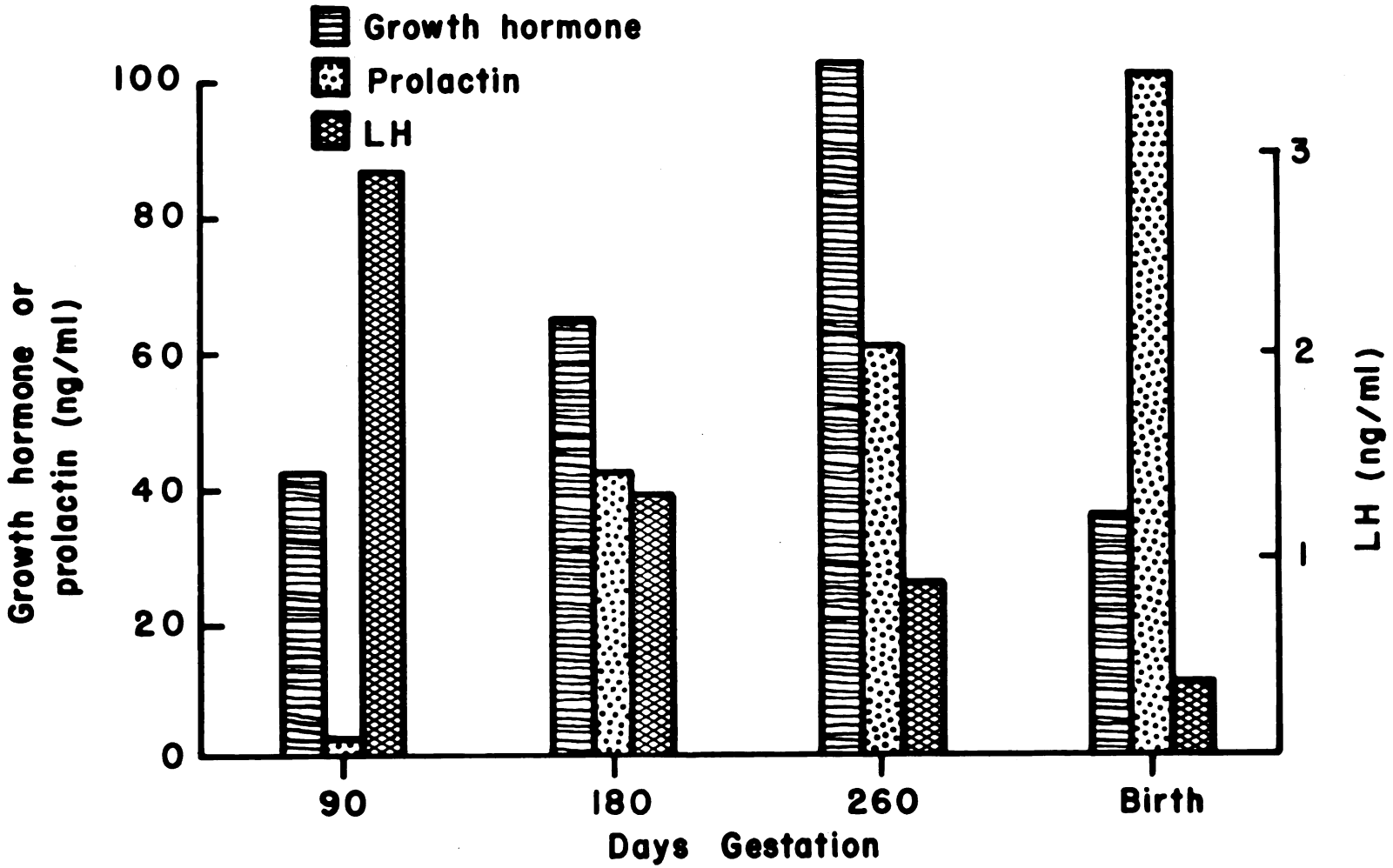


Figure 16 Fetal and neonatal blood serum growth hormone, LH and prolactin.

remained relatively constant strongly indicate that differential hormone control mechanisms are operative during this important period in the life of the fetus. Knowledge of the hormonal levels during fetal development established by this study pave the way for further studies to determine more precisely the role of these hormones in the developing fetus.

Information from this study will serve as the beginning of further studies. Each of us may see new horizons from the platform of knowledge built by preceding researchers (for example Lillie, Jost and Wells). At present, the effect of hormones on the metabolic activity of various tissues and cells are of great interest. The present study should provide a platform to build future studies of fetal hormone action.

SUMMARY AND CONCLUSIONS

Growth hormone (GH), luteinizing hormone (LH) and prolactin were quantified by radioimmunoassay in umbilical artery and umbilical vein at 90, 180 and 260 days of development of 37 bovine fetuses and through the first 6 days after birth of 10 calves. Also blood samples were taken from the jugular vein, uterine artery and vein of each pregnant cow.

Average fetal body weights were 0.5, 6.4 and 26.7 kg for 90, 180 and 260 days of gestation, respectively, and body weight was correlated with fetal age ($r = 0.86$, ($P < 0.01$). Crown-rump length averaged 22.5, 54.0 and 84.4 cm, respectively. The crown-rump lengths were correlated with fetal age and with fetal body weights ($r = 0.97$, $r = 0.93$, respectively, $P < 0.01$). The adrenals, ovaries, uteri, testes and seminal vesicles all increased in weight as gestation lengthened and were correlated with crown-rump lengths ($r = 0.96$, $r = 0.84$, $r = 0.95$, $r = 0.91$, $r = 0.80$, respectively, $P < 0.01$).

Maternal serum GH levels increased from 5.7 ng/ml at 90 days to 10.0 ng/ml at 260 (not significant), but averaged from two to four times higher in the jugular vein than in either of the uterine vessels ($P < 0.01$). At 90,

180 and 260 days, the uterine arterial blood levels of GH were higher than those of the uterine vein, but these differences were not significant. The jugular blood sample from cows with male fetuses averaged significantly higher GH (12.3 ng/ml) at 260 days than the cows with female fetuses (7.3 ng/ml, $P \sim 0.11$). This is the first report of a fetal sex effect on maternal pituitary secretion to my knowledge.

The fetal anterior pituitary GH concentration increased during gestation (4.2, 8.9 and 18.1 µg/mg at 90, 180 and 260 days, respectively, $P < 0.01$). Male and female fetuses had similar pituitary GH at 90 and 180 days, but males averaged 12.1 and females 25.3 µg/mg at 260 days ($P < 0.01$). Serum GH levels increased ($P < 0.01$) with fetal age from 42, to 65 and to 103 ng/ml for 90, 180 and 260 days, respectively, but male and female fetuses did not differ in serum GH. Umbilical arterial and venous serum GH levels were not significantly different.

In the pregnant cows, serum LH levels did not differ in the three blood sources or with stage of gestation, and averaged 0.74 to 0.89 ng/ml.

Fetal pituitary LH concentration increased with fetal age from 323, to 474 and to 535 ng/mg for 90, 180 and 260 days, respectively ($P \sim 0.06$). In contrast, fetal serum LH levels decreased from 3.00, to 1.28 and to 0.85 ng/ml for 90, 180 and 260 days of fetal age,

respectively ($P < 0.01$). Female fetal serum LH averaged 3.90 ng/ml at 90 days, significantly higher than males (1.46 ng/ml) at the same age ($P < 0.01$). Female fetal serum LH also averaged higher at 180 days, but males were higher at 260 days causing a significant sex-age interaction ($P < 0.01$). Levels of serum LH in the umbilical arterial samples were similar to those in the venous samples.

Jugular serum prolactin in cows averaged 220, 145 and 365 ng/ml at 90, 180 and 260 days, respectively; these prolactin levels averaged slightly but not significantly higher than those in the uterine vessels. At 90, 180 and 260 days, cows carrying male fetuses had significantly more (3-, 2- and 7-fold respectively) jugular prolactin than cows carrying females ($P < 0.05$).

Fetal pituitary prolactin concentration increased ($P < 0.01$) with fetal age; 72, 1150 and 2508 ng/mg for 90, 180 and 260 days, respectively, but sex of fetus did not influence fetal pituitary prolactin. The fetal pituitaries synthesized large amounts of prolactin during *in vitro* incubations; up to 23.6 µg/mg during the 72 hour incubation period in 180- and 260-day fetuses. Female pituitaries synthesized three times more prolactin than males at 180 and 260 days ($P \sim 0.07$).

Prolactin levels in the fetal serum averaged 4, 43 and 61 ng/ml for 90, 180 and 260 days of gestation,

respectively ($P < 0.05$). As for GH, prolactin levels in sera from umbilical arteries averaged slightly higher than those from umbilical veins.

At birth serum GH averaged 36 ng/ml and LH averaged 0.36 ng/ml, and neither GH nor LH changed significantly during the first week following birth. However, serum prolactin averaged 101 ng/ml at birth, decreased to an average of 42 ng/ml by the second day after birth ($P < 0.01$), and remained relatively constant to day 6.

The serum hormone gradients maintained by the placenta between the fetus and the cow indicate minimal placental transfer of GH, LH and prolactin and that the fetal pituitary is the principal source of these hormones in fetal serum. Furthermore, age and sex differences in the fetal pituitary hormones, in vitro synthesis of hormones and in fetal serum hormones all indicated that the fetal endocrine system is at least in part controlled independently of the dam. In fact, fetal influences on the maternal endocrine system were also observed in this study.

BIBLIOGRAPHY

BIBLIOGRAPHY

Adams, W. M. and W. C. Wagner. 1970. The role of corticords in parturition. Bio. Reprod. 3: 223-228.

Ainsworth, L. and K. J. Ryan. 1967. Steroid transformations by endocrine organs from pregnant mammals II. Formation and metabolism of progesterone by bovine and sheep placental preparations in vitro. Endocrinol. 81: 1349-1356.

Ainsworth, L. and K. J. Ryan. 1970. Steroid hormone transformations by endocrine organs from pregnant mammals VI. The conversion of Δ^4 androstene-3, 17-dione to estrogens by goat placental preparations in vitro. Steroids. 5: 553-559.

Alleva, F. R., J. J. Alleva and E. J. Umberger. 1969. Effect of a single prepubertal injection of testosterone propionate on later reproductive functions of the female golden hamster. Endocrinol. 85: 312-318.

Almond, C. H., B. M. Boulos, L. E. Davis, and J. W. Mackenzie. 1970. New surgical technique for studying placental transfer of drugs in vivo. J. Surg. Res. 10: 7-11.

Amoroso, E. C. and C. A. Finn. 1962. Ovarian activity during gestation, ovum transport and implantation. In Sir S. Zuckerman, ed., The Ovary, Vol. I, pp. 451-538, Academic Press, New York and London.

Attal, J. Levels of testosterone, androstenedione, estrone and estradiol-17β in the testes of fetal sheep. 1969. Endocrinol. 85: 280-289.

Banting, F. G. and C. H. Best. 1922. Pancreatic extracts. J. Lab. Clin. Med. 7: 464-472.

Barraclough, C. A. 1967. Modifications in reproductive function after exposure to hormones during the prenatal and early postnatal period. In L. Martini and W. F. Ganong, eds., Neuroendocrinology, Vol. II, pp. 61-99, Academic Press, New York and London.

Bascom, K. F. 1923. Interstitial cells in the gonads of cattle with special reference to their embryonic development and significance. Am J. Anat. 31: 223-259.

Bassett, J. M. and G. D. Thorburn. 1969. Foetal plasma corticosteroids and the initiation of parturition in sheep. J. Endocrinol. 44: 285-286.

Bassett, J. M., G. D. Thorburn and A. L. Wallace. 1970. The plasma growth hormone concentration of the foetal lamb. J. Endocrinol. 48: 251-263.

Bates, R. W., O. Riddle, and E. L. Lahr. 1935. Assay of three hormones present in anterior pituitaries of seven types of cattle classified for age, sex and stage of reproduction. Am J. Physiol. 113: 259-264.

Benirschke, K. 1956. Adrenals in anencephaly and hydocephaly. Obstet. Gynecol. 8: 412-425.

Benirschke, K. and E. Bloch. 1960. Failure to demonstrate C_{19}-steroids in bovine testes. Acta Endocrinol. 34: 65-68.

Birge, C. A., G. T. Peake, I. K. Mariz and W. H. Daughaday. 1967. Radioimmunoassayable growth hormone in the rat pituitary gland: effects of age, sex and hormonal state. Endocrinol. 81: 195-204.

Blizzard, R. M. and M. Alberts. 1956. Hypopituitarism, hypoadrenalism and hypogonadism in the newborn infant. J. Pediat. 48: 782-789.

Bouin, P. and P. Ancel. 1903. Recherches sur les cellules interstitielles du testicule des mammiferes. Arch. Zool. Exper. Gen., T. 1, 437-523.

Brauman, J., H. Brauman and J. L. Pasteels. 1964. Immunoassay of growth hormone in cultures of human hypophysis by the method of complement fixation: comparison of the growth hormone secretion and the prolactin activity. Nature (London). 202: 1116-1118.

Brewer, D. B. 1957. Congenital abscence of the pituitary gland and its consequences. J. Pathol. Bacteriol. 73: 59-67.

Brody, S. 1969. Protein hormones and hormonal peptides from the placenta. In A. Klopper and E. Diczfalusy, eds., Foetus and Placenta, pp. 299-411, Blackwell, Oxford and Edinburgh.

Brody, S. and G. Carlström. 1965. Human chorionic gonadotrophin pattern in serum and its relation to the sex of the fetus. J. Clin. Endocrinol. Metabol. 25: 792-797.

Bridges, C. B. 1939. Cytological and genetic basis of sex. In H. Allen, C. H. Danforth and E. A. Doisy, eds., Sex and Internal Secretions, 2nd ed., pp. 15-63, Williams and Wilkins Co., Baltimore.

Catchpole, H. R. and W. R. Lyons. 1934. The gonad-stimulating hormone of pregnant mares. Am. J. Anat. 55: 167-227.

Chaplin, Catherine C. 1917. A microscopic study of the reproductive system of foetal freemartins. J. Exp. Zool. 23: 453-482.

Chez, R. A., D. L. Hutchinson, H. Salazar and D. H. Mintz. 1970. Some effects of fetal and maternal hypophysectomy in pregnancy. Amer. J. Obstet. Gynecol. 108: 643-650.

Chouraqui, J. and J. P. Weniger. 1969. Secretion de corticosteroides par la surrenale embryonnaire de veau cultivee in vitro. Ann. Endocrinol. 30: 533-543.

Chouraqui, J. and J. P. Weniger. 1970. Identification des corticosteroides secretes par les surrenales embryonnaires de veau, lapin et souris cultivees in vitro. Acta Endocrinol. 65: 650-662.

Cole, H. H., G. H. Hart, W. R. Lyons and H. R. Catchpole. 1933. The development and hormonal content of fetal horse gonads. Anat. Rec. 56: 275-293.

Colwill, J. R., J. R. Davis, G. Meschia, E. L. Makowski, P. Beck and F. C. Battaglia. 1970. Insulin-induced hypoglycemia in the ovine fetus in utero. Endocrinol. 87: 710-715.

Comline, R. S. and M. Silver. 1966. The development of the adrenal medulla of the foetal and new-born calf. J. Physiol. (London). 183: 305-340.

Corbin, A. and E. L. Daniels. 1967. Changes in concentration of female rat pituitary FSH and stalk-median eminence follicle stimulating hormone releasing factor with age. Neuroendocrinol. 2: 304-314.

Cupceancu, B., F. Neumann and A. Ulloa. 1969. The influence of some progestogens on the mammary gland of the foetal mouse during the period of initial differentiation. J. Endocrinol. 44: 475-480.

Czyba, J. C., C. Girod et D. Cottinet. 1969. Effet stérilisant sur les petits du hamster doré de l'administration pendant la lactation, de fortes doses d'un composé anovulatoire. Compt. Rend. 163: 1384-1385.

Desjardins, C. and H. D. Hafs. 1968. Levels of pituitary FSH and LH in heifers from birth through puberty. J. Animal Sci. 27: 472-477.

Diczfalusy, E. and S. Mancuso. 1969. Oestrogen metabolism in pregnancy. In A. Klopper and E. Diczfalusy, eds., Foetus and Placenta, pp. 191-248, Blackwell, Oxford and Edinburgh.

Dorfman, R. I. 1967. The antiestrogenic and antiandrogenic activities of progesterone in defense of a normal fetus. Anat. Rec. 157: 547-557.

Drost, M. and L. W. Holm. 1968. Prolonged gestation in ewes after foetal adrenalectomy. J. Endocrinol. 40: 293-296.

Dubois, M. and P. Mauleon. 1969. Mise en évidence par immunofluorescence des cellules a activité gonadotrope LH dans l'hypophyse du foetus de brebis. C. R. Acad. Sci. (Paris). 269: 219-222.

Eguchi, Y. and Y. Morikawa. 1968. Changes in pituitary-gonadal interregulations during perinatal days in the rat. Anat. Rec. 161: 163-170.

Erickson, B. H. 1966. Development and radioresponse of the prenatal bovine ovary. J. Reprod. Fert. 11: 97-105.

Faiman, C., R. J. Ryan, S. J. Zwirek, and M. E. Rubin. 1968. Serum FSH and HCG during human pregnancy and puerperium. J. Clin. Endocrinol. Metabol. 28: 1323-1329.

Fechheimer, N. S., M. S. Herschler and L. O. Gilmore. 1963. Sex chromosome mosaicism in unlike sexed cattle twins. Proc. Int. Cong. Genet. II, Hague I: 265.

Ferreira, A. J. 1969. Prenatal Environment. C. C. Thomas Publisher, Springfield, Ill.

Foster, D. L. 1971. Regulation of luteinizing hormone secretion in the fetal and neonatal lamb. Ph.D Thesis, University of Illinois.

Gailani, S., A. Nussbaum, W. McDougall and W. McLimans. 1970. Studies on hormone production by human fetal pituitary cell cultures. Proc. Soc. Exp. Biol. Med. 134: 27-32.

Gey, G. O., G. E. Seegar and L. M. Hellman. 1938. The production of a gonadotrophic substance (Prolan) by placental cells in tissue culture. Science. 88: 306-307.

Gier, H. T. and G. B. Marion. 1969. Development of Mammalian testes and genital ducts. Bio. Reprod. Supplement I; June: 1-23.

Gitlin, D. and A. Biasucci. 1969 Ontogenesis of immuno-reactive growth hormone, follicle-stimulating hormone, thyroid stimulating hormone, chorionic prolactin and chorionic gonadotropin in the human conceptus. J. Clin. Endocrinol. Metabol. 29: 926-935.

Gitlin, D., J. Kumate and C. Morales. 1965. Metabolism and maternofetal transfer of human growth hormone in the pregnant women at term. J. Clin. Endocrinol. Metabol. 25: 1599-1608.

Goodman, L. and G. B. Wislocki. 1933. Note on the failure of anterior lobe extract to pass from mother to fetus in rabbits and cats. Am. J. Physiol. 106: 323-328.

Gorski, R. A. 1968. Influence of age on the response of paranatal administration of a low dose of androgen. Endocrinol. 82: 1001-1004.

Grumbach, M. M., S. L. Kaplan, J. J. Sciarra and I. M. Burr. 1968. Chorionic growth hormone-prolactin (CGP): secretion, disposition, biologic activity in man, and postulated function as the "growth hormone" of the second half of pregnancy. Ann. N. Y. Acad. Sci. 148: 501-531.

Gustafsson, P. O. and C. G. Beling. 1969. Estradiol-induced changes in beagle pups: Effect of prenatal and postnatal administration. Endocrinol. 85: 481-491.

Halász, B. and R. A. Gorski. 1967. Gonadotrophic hormone secretion in female rats after partial or total interruption of neural afferents to the medial basal hypothalamus. Endocrinol. 80: 608-622.

Harris, G. W. and D. Jacobsohn. 1951-1952. Functional grafts of the anterior pituitary gland. Proc. Royal Soc. (London) B. 139: 263-276.

Harris, G. W. and S. Levine. 1965. Sexual differentiation of the brain and its experimental control. J. Physio. 181: 379-400.

Hatakeyama, S. 1969. Electron microscope study of the anencephalic adenohypophysis with reference to the adrenocorticotrophs and their correlation with the functional differentiation of the hypothalamus during foetal life. Endocrinol. Japonica. 16: 187-203.

Heggestad, C. B. and L. J. Wells. 1965. Experiments on the contribution of somatotrophin to prenatal growth in the rat. Acta Anat. 60: 348-361.

Hellbaum, A. A. 1935. The gonad-stimulating activity of pituitary glands from horses of different ages and sex types. Anat. Rec. 63: 147-157.

Hernandez, M. 1971. Prenatal and postnatal development of thyroid function in the bovine. M. S. Thesis, Michigan State University.

Hoffman, B. and R. P. Martin. 1968. In vitro studies of steroid biosynthesis in gonads of the freemartin. 6th Cong. Reprod. & A. I. Paris, p. 142.

Hogben, L. T. and E. A. E. Crew. 1923-1924. Studies on internal secretion II endocrine activity in foetal and embryonic life. J. Exptl. Bio. (Brit.). 1: 1-13.

Hunter, W. M. 1967. The preparation of radioidinated proteins of high activity, their reaction with antibody in vitro: The radioimmunoassay. In D. M. Wier, ed., Handbook of Experimental Immunology, p. 608-654. F. A. Davis Co., Philadelphia, Pa.

Hurst, V. 1962. The effects of injecting testosterone into pregnant cows on the reproductive organs of their heifer calves. Proc. Ass. So. Agric. Wkrs. 59th Annual Conv. p. 123.

Hutchinson, D. L., J. L. Westover and D. W. Will. 1962. The destruction of the maternal and fetal pituitary glands in subhuman primates. Am. J. Obstet. Gynecol. 83: 857-865.

Jackson, B. T. and R. H. Egdahl. 1960. The performance of complex fetal operations in utero without amniotic fluid loss or other disturbances of fetal-maternal relationships. Surgery. 48: 564-570.

Jaffe, R. B., P. A. Lee and A. R. Midgley, Jr. 1969. Serum gonadotropins before, at the inception of and following human pregnancy. J. Clin. Endocrinol. Metabol. 29: 1281-1283.

Jainudeen, M. R. and E. S. E. Hafez. 1965. Attempts to induce bovine freemartinism experimentally. J. Reprod. Fert. 10: 281-283.

Jean, C. 1969. Evolution post-natal des anomalies mammaries produites chez les descendants par un traitment oestrogenique de la souris gestante. Comp. Rend. 163: 1126-1131.

Jean, Ch. and P. Delost. 1969a. Androgenes et embryogenese mammaire. J. de Physiol. Suppl. 1. 61: 139-140.

Jean, Ch. and P. Delost. 1969b. Prolactine et embryogenese mammaire chez la Souris. J. de Physio. Suppl. 2. 61: 323.

Jones, H. W. and L. Wilkins. 1960. The genital anomaly associated with prenatal exposure to progestogens. Fert. Steril. 11: 148-156.

Jost, A. 1947. Experiences de décapitation de l'embryon de lapin. C. R. Acad. Sci. (Paris) Ser. D. 225: 322-324.

Jost, A. 1955. Modalities in the action of gonadal and gonad-stimulating hormones in the foetus. Memoirs of the Society for Endocrine. 4: 237-247.

Jost, A. 1965. Gonadal hormones in the sex differentiation of the mammalian fetus. In R. L. DeHaan and H. Ursprung, eds., Organogenesis, pp. 611-628, Holt, Rinehart and Winston, Inc., New York.

Jost, A. 1966. Anterior pituitary function in foetal life. In G. Harris and B. Donovan, eds., The Pituitary Gland. Vol. 2, pp. 299-323. University of California Press.

Jost, A., M. Chodkiewicz and P. Mauleon. 1963. Intersexualité du foetus de veau produite par des androgenes. Comparaison entre l'hormone foetale responsable du freefmartinisme et l'hormone testiculaire adulte. C. R. Acad. Sci. 256: 274-276.

Jost, A. and M. G. Moreau-Stinnakre. 1970. Action d'une substance progestative synthetique (17α-allyl-4-oestrene-17β-ol) sur la differenciation sexuelle des foetus de rat. Remarques methodologies. Acta Endocrinol. 65: 29-49.

Jost, A. and L. Picon. 1970. Hormonal control of fetal development and metabolism. In R. Levine and R. Luft, eds., Advances in Metabolic Disorders, Vol. 4, pp. 123-184, Academic Press.

Karg, H. 1967a. Bestimmung des interstitialzellen stimulierenden hormones in rinderhypophysen wahrend der fetalzeit und der geburt. 13. Symposion der Deutschen Gesellschaft fur Endokrinologie, Wurzburg, pp. 141-143.

Karg, H. 1967b. Untersuchungen uber das gonadotrope hypophysenhormon LH (ISCH) wahrend der entwicklung beim rind. Zuchthygiene, 2: 12-19.

Keller, K. and J. Tandler. 1916. Uber das verhalten der eihaüte bei der zwillingsstrachtigkeit des rindes. Untersuchungen über die entstehungsursache der geschlechtlichen unterentwicklung von weiblichen zwillingskälbern welche neben einem männlichen kalbe zur entwicklung gelangen. Wien. Tierarztl. Wochschr. 3: 513-526.

Kennedy, P. C., J. W. Kendrick and C. Stormont. 1957. Adenohypophysealaplasia, an inherited defect associated with abnormal gestation in Guernsey cattle. Cornell Vet. 47: 160-178.

Kitchell, R. L. and L. J. Wells. 1952. Reciprocal relation between the hypophysis and adrenals in fetal rats: effects of unilateral adrenalectomy and of implanted cortisone, DOCA and sex hormones. Endocrinol. 50: 83-93.

Koch, F. C. 1931. The extraction, distribution and action of testicular hormones. J. Am. Med. Assoc. 96: 937-939.

Kraner, K. L. and C. J. Parshall, Jr. 1969. Experimental procedures and surgical techniques performed on intrauterine fetal animals. In W. I. Gay, ed., Methods of Animal Experimentation. Vol. II, pp. 211-239, Academic Press.

Krehbiel, E. B. 1963. Differentiation of gonads in the bovine embryo. Diss. Abstr. 24: 1761.

Levina, S. E. 1968. Endocrine features in development of human hypothalamus, hypophysis and placenta. Gen. & Comp. Endocrinol. 11: 151-159.

Levine, S. 1971. Sexual differentiation: the development of maleness and femaleness. Calif. Med. 114: 12-17.

Liggins, G. C. and P. C. Kennedy. 1968. Effects of electrocoagulation of the foetal lamb hypophysis on growth and development. J. Endocrinol. 40: 371-381.

Liggins, G. C., P. C. Kennedy and L. W. Holm. 1967. Failure of initiation of parturition after electrocoagulation of the pituitary of the fetal lamb. Amer. J. Obstet. & Gynec. 98: 1080-1086.

Lillie, F. R. 1916. The theory of the freemartin. Science. 43: 611-613.

Lillie, F. R. 1917. The freemartin: a study of the action of sex hormones in the fetal life of cattle. J. Exptl. Zool. 23: 371-452.

Lyons, W. R. 1958. Hormonal synergism in mannary growth. Proc. Royal Soc. Biol. Sci. (B). 149: 303-325.

Lyons, W. R. 1937. The hormonal basis for "witches milk." Proc. Soc. Exp. Biol. Med. 37: 207-209.

MacMillan, D. R. 1970. Endocrine influences on fetal growth. Pediat. Clin. N. Amer. 17: 111-117.

Macmillan, K. L. and H. D. Hafs. 1968. Pituitary and hypothalamic endocrine changes associated with reproductive development of Holstein bulls. J. Animal Sci. 27: 1614-1620.

Macnaughton, M. C. 1969. Endocrinology of the foetus. In A Klopper and E. Diczfalusy, eds., Foetus and Placenta, pp. 557-602, Blackwell, Oxford and Edinburgh.

Mason, R. W., J. E. Bone, R. Bogart and H. Krueger. 1958. Urogenital anomalies in calf born to beef cow treated with testosterone during pregnancy. J. Pharm. & Exptl. Ther. 122: 49.

Matschke, G. H. and B. H. Erickson. 1969. Development and radioresponse of the prenatal bovine testis. Bio. Reprod. 1: 207-214.

Mauleon, P. and M. M. Reviers. Variations avec l'age et le sexe des teneurs en hormones folliculo-stimulante (FSH) et luteinisante (LH) des antéhypophyses de foetus de mouton. Ann. Biolog. Anim. Bioch. Biophys. 9: 475-481.

McClung, C. E. 1902. The accessory chromosome--sex determinant? Biol. Bull. 3: 43-84.

McCord, Carey Pratt. 1915. The occurence of pituitrine and epinephrine in foetal pituitary and suprarenal glands. J. Biol. Chem. 23: 435-438.

Meites, J., R. H. Kahn and C. S. Nicoll. 1961. Prolactin production by rat pituitary explants in vitro. Proc. Soc. Exp. Biol. Med. 180: 440-443.

Melampy, R. M., D. M. Hendricks, L. L. Anderson, C. L. Chen and J. R. Schultz. 1966. Pituitary follicle-stimulating hormone and luteinizing hormone concentrations in pregnant and lactating pigs. Endocrinol. 78: 801-804.

Mellor, D. J. 1969. Vascular anastomosis and fusion of foetal membranes in multiple pregnancy in the sheep. Res. Vet. Sci. 10: 361-367.

Meneghelli, V. and R. Scapinelli. 1962. The site and time of onset of growth hormone production in the bovine adenohypophysis, determined by the fluorescent antibody method. Acta Anat. 51: 198-208.

Mitskevich, M. S. 1962. Hormonal interrelationships between mother and fetus in mammals. Gen. Comp. Endocrinol. Suppl. 1: 300-308.

Morrison, F. B. 1957. Feeds and Feeding, 22nd ed. Morrison Publishing Co., Ithaca, New York, p. 680.

Nanagas, J. C. 1925. A comparison of the growth of the body dimensions of anencephalic human fetuses with normal fetal growth as determined by graphic analysis and empirical formulae. Am. J. Anat. 35: 455-494.

Neumann, F., W. Elger and H. Steinbeck. 1969. Drug-induced intersexuality in mammals. J. Reprod. & Fert. Supp. 7: 9-24.

Niswender, G. D., L. E. Reichert, Jr., A. R. Midgley, Jr. and A. V. Nalbandov. 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinol. 84: 1166-1173.

Ohno, S. 1969. The problem of the bovine freemartin. J. Reprod. & Fert. Supp. 7: 53-61.

Parshall, C. J. and A. M. Silverstein. 1969. Surgical approaches to the study of fetal immunology in primate animals. Ann. N. Y. Acad. Sci. 147: 254-266.

Pasqualini, J. R., B. L. Nguyen, F. Uhrich, N. Wiqvist and E. Diczfalusy. 1970. Cortisol and cortisone metabolism in the human foeto-placental unit at midgestation. J. Steroid Bioch. 1: 209-219.

Pavlova, E. B., T. S. Pronina and Y. B. Skebelskaya. 1968. Histostructure of adenohypophysis of human fetuses and contents of somatotrophic and adrenocorticotrophic hormones. Gen. Comp. Endocrinol. 10: 269-276.

Piacsek, B. E. and J. Meites. 1967. Reinitiation of gonadotropin release in underfed rats by constant light or epinephrine. Endocrinol. 81: 535-541.

Pierrepoint, C. G., A. B. M. Anderson, K. Griffiths, and A. C. Turnbull. 1969a. Metabolism of C_{19}-steroids by foetal cotyledons from the bovine placenta at term. Res. Vet. Sci. 10: 477-476.

Pierrepoint, C. G., J. S. Stewart and J. Rack. 1969b. Patterns of steroid biosynthesis in the gonads of freemartins and their male co-twins. J. Reprod. & Fert. Supp. 7: 63-72.

Price, D. and R. Pannabecker. 1959. Comparative responsiveness of homologous sex ducts and accessory glands of foetal rats in culture. Arch. Anat. Microsc. Morph. Exp. 48 bis: 223-243.

Purchas, R., K. Macmillan and H. Hafs. 1970. Pituitary and plasma growth hormone levels in bulls from birth to one year of age. J. Animal Sci. 31: 358-363.

Rawlings, W. J. and V. I. Krieger. 1964. Pregnanediol excretion and sex of the fetus. Fert. & Steril. 15: 173-179.

Raud, H. R., C. A. Kiddy and W. D. Odel. 1971. The effect of stress upon the determination of serum prolactin by radioimmunoassay. Proc. Soc. Exp. Biol. Med. 136: 689-693.

Reece, R. P. and C. W. Turner. 1937. The lactogenic and thyrotropic content of the anterior lobe of the pituitary gland. Missouri Agr. Exper. Stat. Res. Bull. 266.

Resko, J. A. 1970. Androgen secretion by the fetal and neonatal rhesus monkey. Endocrinol. 87: 680-687.

Rice, B. F., R. Ponthier, Jr., and W. Sternberg. 1968. Luteinizing hormone and growth hormone activity of the human fetal pituitary. J. Clin. Endocrinol. Metabol. 28: 1071-1072.

Roberts, J. D. and J. C. Warren. 1964. Steroid biosynthesis in the fetal ovary. Endocrinol. 74: 846-852.

Roberts, S. J. 1956. Veterinary obstetrics and genital diseases. Dist. by Eward Bros., Ann Arbor, Mich.

Schams, D. 1969. Radioimmunobiologische bestimmung des luteinisierungshormons (LH) im blutserum von kühen in den ersten zwei monaten der trachtigkeit. Dtsch. tierarztl. wschr. 76: 567-569.

Schams, D. and H. Karg. 1970. Untersuchungen über prolactin im rinderblut mit einer radioimmunologischen bestimmungsmethode. Zbl. Vet. Med. A, 17: 193-212.

Schul, G., L. Smith, L. Goyings and R. Zimbleman. 1970. Effects of oral melengestrol acetate (MGA) on the pregnant heifer and on her resultant offspring. J. Animal Sci. 30: 433-437.

Shane, B. S., Dunn, R. M. Kenney, W. Hansel and W. J. Visek. 1969. Methyl testosterone-induced female pseudohermaphroditism in dogs. Bio. Reprod. 1: 41-48.

Shemesh, M., N. Ayalon, and H. R. Linder. 1968. Early effect of conceptus on plasma progesterone level in the cow. J. Reprod. & Fert. 15: 161-164.

Short, R. V. 1969. An introduction to some of the problems of intersexuality. J. Reprod. & Fert. Supp. 7: 53-61.

Smith, C. A. 1959. The physiology of the newborn infant. 3rd ed., pp. 365-397. C. C. Thomas, Springfield, Ill.

Smith, J. A. and W. D. Ross. 1910. The works of Aristotle translated into English. IV. Historia animalum. Clarendon Press, Oxford.

Smith, P. E. and C. Dortzbach. 1929. The first appearance in the anterior pituitary of the developing pig foetus of detectable amounts of the hormones stimulating ovarian maturity and general body growth. Anat. Rec. 43: 277-297.

Snow, H. D. and J. G. Tyner. 1969. Chronic arterial and venous catheterization in sheep. J. Amer. Vet. Res. 30: 2241-2243.

Stabenfeldt, G. H., L. L. Ewing, J. P. Patton and L. E. McDonald. 1969. Gas-liquid chromatography for estimation of peripheral plasma progesterone in domestic animals. J. Endocrinol. 44: 23-38.

Stewart, J. S. S. 1965. The clinical investigation of intersexuality. Ann. R. Coll. Surg. 37: 374-388.

Stokes, H. and J. M. Boda. 1968. Immunofluorescent localization of growth hormone and prolactin in the adenohypophysis of fetal sheep. Endocrinol. 83: 1362-1366.

Struck, H. and H. Karg. 1967. Bestimmung von testosteron und androstendion in rinderhoden wahrend der fetalzeit und der geburt. 13. Symposion der Deutschen Gesellschaft fur Endokrinologie Wurzburg, p. 141-143.

Swanson, L. 1970. Endocrine, behavioral, and ovarian changes in Holstein heifers from puberty to breeding size. Ph.D. Thesis, Michigan State University.

Sweat, M. L., D. L. Berliner, M. J. Bryson, C. Naboss, J. Haskell and E. G. Holstrom. 1960. The synthesis and metabolism of progesterone in the human and bovine ovary. Biochem. Biophys. Acta 40: 289-296.

Swenson, E. A. 1925. The use of cerebral anemia in experimental embryological studies upon mammals. Anat. Rec. 30: 147-151.

Tucker, H. A. 1971. Hormonal response to milking. J. Animal Sci. 32: Suppl. I, 137-141.

Van Wagenen, G. and M. E. Simpson. 1954. Testicular development in the rhesus monkey. Anat. Rec. 118: 231-251.

Wells, L. J. 1946. Effects of injections of equine gonadotrophin upon the gonads and adrenals of fetal rats. Proc. Soc. Exp. Biol. Med. 62: 250-254.

Wells, L. J. 1947. Progress of studies designed to determine whether the fetal hypophysis produces hormones that influence development. Anat. Rec. 97: 409.

Willier, B. H. 1921. Structure and homologies of free-martin gonads. J. Exptl. Zool. 33: 63-127.

Winters, L., W. Green and R. Comstock. 1942. Prenatal development of the bovine. Univ. Minn. Agric. Exp. Station Tech. Bull. 151.

Yalow, R. S. and S. A. Berson. 1968. General principles of radioimmunoassay. In R. L. Hayes, F. A. Goswitz and B. E. P. Murphy, eds., Radioisotopes in Medicine: In Vitro Studies., pp. 7-39. U. S. Atomic Energy Commission.

Yamuchi, S. and F. Sasaki. 1968. Studies on the vascular supply to the uterus of the cow. I. Morphological studies of arteries in a broad ligament. Bull. Univ. Osaka Pref. Ser. 20: 33-47.

Yamuchi, S. and F. Sasaki. 1969. Studies on the vascular supply of the uterus of a cow. III. Morphological studies of veins in the broad ligament. Jap. J. Vet. Sci. 31: 9-22.

Young, W. C. 1961. The hormones and mating behavior. In W. C. Young and G. W. Corner, eds., Sex and Internal Secretions, pp. 1173-1239, Williams and Wilkins Co., Baltimore.

Young, W. C. 1966. The organization of sexual behavior by hormonal action during the prenatal and larval periods in vertebrates. In F. A. Beach, ed., Sex and Behavior. p. 89, J. Wiley and Sons, New York.

Younglai, E. V. and S. Solomon. 1969. Neutral steroids in human pregnancy: isolation, formation and metabolism. In A. Klopper and E. Diczfalusy, eds., Foetus and Placenta, pp. 249-298, Blackwell, Oxford and Edinburgh.

Zapol, W. M., T. Kolobow, J. E. Pierce, G. G. Vurek and R. L. Bowman. 1969. Artificial placenta: two days of total extrauterine support of the isolated premature lamb fetus. Science. 166: 617-618.

Zelenik, J. S. 1965. Endocrine physiology of pregnancy. Clin. Obstet. Gynec. 8: 528-549.

Zimbleman, R. G. 1964. Injection of steroids into newborn farm animals. J. Animal Sci. 23: 1230.

Zondek, L. H. and T. Zondek. 1965. Observations on the testes in anencephaly with special reference to the leydig cells. Biol. Neonatorium. 8: 329-347.

APPENDICES

APPENDIX

Appendix I. Composition of reagents used in radioimmunoassay.

A. Reagents for radioiodination

1. 0.5 M sodium phosphate buffer, pH 7.5
   Monobasic (0.5 M)
   Add 69.005 g $NaH_2PO_4.H_2O$ to distilled water.
   Dissolve, dilute to 1 liter.
   Dibasic (0.5 M)
   Add 70.98 g Na_2HPO_4 to distilled water.
   Heat to dissolve, then dilute to 1 liter.
   Mix monobasic and dibasic to give pH 7.5.
   Dispense in 1 ml portions, store at -20°C.
   Store the monobasic and dibasic buffers at 4° C.

2. 0.05 M sodium phosphate buffer, pH 7.5
   Solution A
   $NaH_2PO_4.H_2O$ ----------2.78 g
   Merthiolate ----------0.01 g
   Dilute to 100 ml with distilled water.
   Solution B
   $NaHPO_4/7H_2O$ ----------26.825 g
   Merthiolate ---------- 0.05 g
   Dilute to 500 ml with distilled water.
   Use 16 ml Solution A, 84 ml Solution B, dilute to 400 ml with distilled water.
   Adjust pH to 7.5 with NaOH, if necessary.
   Store at 4° C.

3. Chloramine-T
   Upon receiving chloramine-T, dispense into small, tightly sealed vials, cover with foil, and store at -20° C.
   Dilute 10 mg* chloramine-T to 10 ml with 0.05 M $NaPO_4$, pH 7.5 buffer. Use within 30 minutes of preparation. Discard chloramine-T remaining in vial.
   *30 mg for GH

4. Sodium metabisulfite, 2.5 µg/µl
   Dilute 25 mg $Na_2S_2O_5$ to 10 ml with 0.05 M NaPO4, pH 7.5 buffer. Use within 30 minutes of preparation.

5. Transfer solution
   Sucrose----------1.6 g
   KI ----------0.1 g
   Dilute to 10 ml with distilled water.
   Dispense in 1 ml portions, store at -20° C.

6. Rinse solution
   Sucrose ---------- 0.8 g
   KI---------- 0.1 g
   Bromphenol blue---------- 0.001 g
   Dilute to 10 ml with distilled water.
   Dispense in 1 ml portions, store at -20° C.

B. Reagents for Radioimmunoassay

1. 0.01 M phosphate buffered saline, pH 7.0 (PBS)
   NaCl---------- 143 g
   Monobasic phosphate---------- 100 ml
   (See Appendix A.1)
   Dibasic phosphate---------- 260 ml
   (See Appendix A.1)
   Merthiolate---------- 1.75 g
   Dissolve in distilled water and transfer to a large container.
   Dilute to 17.5 liters with distilled water.
   Adjust pH to 7.0, if necessary, store at 4° C.

2. 0.05 M EDTA - PBS, pH 7.0
   disodium EDTA---------- 18.612 g
   Add approximately 950 ml PBS.
   Adjust pH to 7.0 with 5 NaOH while stirring.
   Dilute to 1 liter, store at 4° C.

3. PBS - 1% egg white albumin (PBS - 1% EWA) or PBS - 1% bovine serum albumin (PBS - 1% BSA).
   Add 990 ml PBS to beaker.
   Add 10 g EWA (Sigma Chemical Corp.) or 10 g BSA.
   Mix over magnetic mixer.
   Filter through Whatman No. 1 filter paper.
   Store at 4° C.

4. Hormone standards (LH, GH and prolactin)
   PBS - 1% EWA is used for LH and PBS - 1% BSA is used for GH and prolactin; hereafter they will be referred to as buffers.
   Rinse a small screw-cap vial with buffer, dry.
   Weigh 200-400 ug NIH-LH-B5, NIH-GH-B12 or NIH-P-B1 on Cahn Electrobalance and transfer hormone to the screw-cap vial.
   Add 0.85% saline to 1 mg/ml.
   Add buffer to 9 volumetric flasks.
   Using Hamilton microliter syringes, add appropriate volumes of the 1 mg/ml stock hormone to volumetric flasks to obtain the following concentrations:
   LH - 0.16, 0.32, 0.64, 1.28, 2.56, 5.12, 10.24, 20.48 and 40.96 ng/ml.
   GH - 0.2, 0.6, 1.0, 1.6, 2.0, 3.0, 4.0, 6.0, 8.0 and 10.0 ng/ml.
   Prolactin - 0.2, 0.4, 1.0, 1.6, 2.0, 3.0, 4.0, 6.0 and 8.0 ng/ml.

Add buffer to final volume in each volumetric flask.
Dispense each standard in quantities suitable for one assay.
Freeze in Dry Ice - ethanol, store at -20° C.
For use, thaw rapidly with 38° C. water.

5. 1:400 normal guinea pig serum (NGPS).
   Obtain blood from guinea pig that has not been used to develop antibodies.
   Allow blood to clot, recover serum and store the serum in convenient quantities at -20° C.
   Add 2.5 ml of appropriate serum to a 1 liter volumetric flask, dilute to 1 liter with 0.05 M PBS-EDTA, pH 7.0
   Divide into 100-ml portions and store at -20° C.

6. Guinea pig anti-bovine LH (GPABLH, identified in our laboratory as antibody I), guinea pig anti-bovine GH (GPABGH), or guinea pig anti-bovine prolactin (GPABP).
   Dilute the antisera to 1:400 with 0.05 M PBS-EDTA, pH 7.0.
   Dispense in small quantities, store at -20° C.
   On day of use, dilute the 1:400 antisera to the required concentration using 1:400 NGPS as diluent.

7. Anti-gamma globulin
   Use goat anti-guinea pig gamma globulin (GAGPGG) in LH assay and sheep anti-guinea pig gamma globulin (SAGPGG) in GH and prolactin assay.
   Dilute antisera to required concentration with 0.05 M PBS-EDTA, pH 7.0.
   Store at 4° C. or at -20° C.

C. Antibody and anti-gamma globulin production

1. Guinea pig anti-LH
   0.5 or 1.0 mg NIH-LH-B5 was dissolved in water and Freund's complete adjuvant added (1:1 ratio).
   1.1 or 0.6 ml of the emulsion per guinea pig was injected subcutaneously in 4 scapular region sites.
   The above procedure was repeated 15 and 30 days later substituting Freund's incomplete adjuvant for adjuvant.
   Antisera was collected by cardic puncture 46 and 78 days after the initial injection.

2. Goat anti-guinea pig gamma globulin
   Guinea pig gamma globulin (Fraction 11, Pentex, Inc., Kankakee, Illinois) (40 mg), streptomycin (100 mg) and penicillin (1000 I.U.) was emulsified in 5 ml of water plus 5 ml Freund's complete adjuvant.
   10 ml was subcutaneously injected in 8 scapular sites of a 75 kg goat.
   The above procedure repeated 15 days later substituting Freund's incomplete adjuvant for adjuvant.
   Antisera was collected 30 days after the second antigen injection by jugular vein puncture.

Appendix II. Maternal physical and hormonal data on cows at 90 days gestation.

Sex of fetus	Cow number	Cow weight	Serum hormone data								
			GH			LH			Prolactin		
			Jugular vein	Uterine artery	Uterine vein	Jugular vein	Uterine artery	Uterine vein	Jugular vein	Uterine artery	Uterine vein
		(kg)	(ng/ml)								
Male	544	383	12.5	3.0	2.3	0.8	0.8	0.8	1480	492	390
	548	353	3.8	1.6	1.2	0.8	0.8	0.8	73	153	146
	552	309	8.0	2.3	2.5	0.6	0.7	0.6	301	137	143
	576	375	3.3	4.7	2.6	1.1	0.8	1.0	17	75	30
	577	400	2.4	1.9	2.1	0.8	0.7	0.9	20	26	7
	Mean	364	6.0	2.7	2.1	0.82	0.76	0.82	378	176	143
	S.E.	16	1.8	0.6	0.3	0.08	0.02	0.07	280	82	68
	n	5	5	5	5	5	5	5	5	5	5
Female	553	297	16.7	2.2	2.2	0.7	0.7	0.8	572	668	186
	570	425	2.2	3.2	1.4	0.9	0.7	1.0	32	48	30
	571	388	5.8	1.3	1.2	0.7	0.8	0.8	86	51	63
	572	397	2.8	2.0	1.7	0.8	0.9	0.7	68	64	43
	573	345	3.0	1.7	1.9	1.0	1.0	0.9	47	138	29
	574	377	3.7	1.7	1.1	0.7	1.5	0.6	13	66	42
	575	352	7.8	1.4	1.1	0.8	0.9	0.9	129	94	94
	578	452	2.3	2.2	1.6	1.0	1.3	1.1	17	59	46
	Mean	379	5.5	2.0	1.5	0.83	0.98	0.85	120	148	67
	S.E.	17	1.7	0.2	0.1	0.05	0.10	0.06	66	75	19
	n	8	8	8	8	8	8	8	8	8	8
Mean		373	5.7	2.2	1.8	0.82	0.89	0.84	220	159	96
S.E.		12	1.3	0.3	0.2	0.04	0.07	0.04	114	54	29
n		13	13	13	13	13	13	13	13	13	13

Appendix III. Maternal physical and hormonal data in cows at 180 days gestation.

Sex of fetus	Cow number	Cow weight	Serum hormone data								
			GH			LH			Prolactin		
			Jugular vein	Uterine artery	Uterine vein	Jugular vein	Uterine artery	Uterine vein	Jugular vein	Uterine artery	Uterine vein
		(kg)	(ng/ml)								
Male	540	366	3.8	3.3	3.3	0.8	0.8	0.8	56	58	37
	542	388	34.1	1.7	1.5	0.7	0.7	0.7	996	624	324
	554	359	6.5	2.0	2.3	0.7	0.6	0.7	31	23	22
	555	353	4.0	2.8	2.2	0.7	0.6	0.6	39	436	484
	556	432	5.4	4.9	2.2	0.8	0.7	0.7	32	77	48
	558	361	16.9	2.5	2.1	1.2	0.8	1.0	148	13	11
	559	380	4.9	1.4	1.8	0.7	2.0	0.8	68	11	8
	Mean	377	10.8	2.6	2.2	0.80	0.88	0.75	196	177	133
	S.E.	10	4.2	0.4	0.2	0.07	0.18	0.04	134	94	72
	n	7	7	7	7	7	7	7	7	7	7
Female	541	404	8.8	2.8	2.1	0.7	0.7	0.7	297	156	130
	546	374	3.8	2.1	2.4	0.8	0.8	0.7	53	123	51
	547	391	2.8	1.5	2.0	0.7	0.7	0.7	22	57	29
	551	360	3.2	2.3	2.4	1.1	0.9	0.9	19	259	171
	557	436	3.6	9.2	4.8	0.9	0.9	1.0	56	310	182
	567	400	4.1	2.0	3.3	1.2	0.9	0.9	66	73	37
	Mean	394	4.4	3.3	2.8	0.90	0.81	0.81	85	163	100
	S.E.	11	0.9	1.2	0.4	0.08	0.04	0.05	43	42	28
	n	6	6	6	6	6	6	6	6	6	6
Mean		385	7.8	3.0	2.5	0.85	0.85	0.78	145	171	118
S.E.		8	2.4	0.6	0.2	0.05	0.10	0.03	74	52	40
n		13	13	13	13	13	13	13	13	13	13

Appendix IV. Maternal physical and hormonal data in cows at 260 days gestation.

Sex of fetus	Cow number	Cow weight	Serum hormone data								
			GH			LH			Prolactin		
			Jugular vein	Uterine artery	Uterine vein	Jugular vein	Uterine artery	Uterine vein	Jugular vein	Uterine artery	Uterine vein
		(kg)	(ng/ml)								
Male	515	600	4.7	1.9	1.1	0.7	0.7	0.7	172	716	192
	524	490	7.7	1.6	2.0	0.6	0.7	0.6	89	288	252
	539	445	26.0	2.9	3.7	0.7	0.7	0.7	1991	308	376
	549	374	28.1	1.8	1.6	0.6	0.6	0.7	716	162	196
	562	399	2.8	1.7	2.5	0.8	0.8	0.7	148	19	15
	566	441	4.8	1.3	1.1	1.1	0.8	0.9	460	38	32
	Mean	458	12.3	1.9	2.0	0.75	0.72	0.72	596	255	177
	S.E.	33	4.7	0.2	0.4	0.07	0.03	0.04	295	105	58
	n	6	6	6	6	6	6	6	6	6	6
Female	525	468	1.6	1.3	1.8	0.7	0.6	0.6	42	530	332
	550	423	2.6	1.8	1.8	1.0	1.1	1.1	184	131	220
	560	445	1.8	2.0	0.6	0.6	0.8	0.8	24	17	20
	563	440	3.5	2.1	2.3	0.7	1.8	0.6	38	22	7
	564	415	27.0	2.1	1.7	0.7	0.7	0.7	148	51	36
	Mean	438	7.3	1.9	1.6	0.74	1.00	0.76	87	150	123
	S.E.	9	5.0	0.1	0.3	0.07	0.21	0.09	33	97	65
	n	5	5	5	5	5	5	5	5	5	5
Mean		449	10.1	1.9	1.8	0.74	0.85	0.74	365	207	153
S.E.		18	3.3	0.1	0.2	0.05	0.10	0.04	174	70	41
n		11	11	11	11	11	11	11	11	11	11

Appendix V. Physical and hormonal characteristics of fetuses at 90 days gestation.

Sex	Cow (or fetal) number	Age	Body weight	Crown-Rump length	Adrenal weight	Paired testicular or ovarian weight	Seminal vesicular or uterine weight
		(days)	(kg)	(cm)	(mg)	(mg)	(mg)
Male	544	91	0.280	19.1	81	91	--
	548	89	0.398	21.6	105	116	--
	552	88	0.383	21.6	60	93	--
	576	93	0.560	24.1	146	195	66
	577	91	1.050	26.7	229	161	150
	Mean	90.4	0.534	22.6	124	131	--
	S.E.	0.9	0.136	1.3	30	20	--
	n	5	5	5	5	5	--
Female	553	88	0.400	22.2	104	81	168
	570	91	0.425	21.6	71	64	114
	571	91	0.520	23.9	102	78	159
	572	91	0.440	22.9	135	122	98
	573	91	0.220	19.1	58	71	77
	574	91	0.475	22.2	88	88	156
	575	93	0.345	22.9	78	87	98
	578	92	0.640	24.1	146	72	171
	Mean	91	0.433	22.3	98	83	130
	S.E.	0.5	0.044	0.6	11	6.3	13
	n	8	8	8	8	8	8
Mean		90.7	0.472	22.5	108	--	--
S.E.		0.4	0.057	0.6	13	--	--
n		13	13	13	13	--	--

Appendix V. Con't.

Systemic blood serum hormones			Anterior pituitary hormone content		
GH	LH	Prolactin	GH	LH	Prolactin
(ng/ml)			(ng/mg)		
20	1.7	4.7	4	6	13
30	1.6	2.5	2870	236	52
22	0.9	3.7	4305	214	103
40	1.7	3.7	8980	553	235
36	1.4	2.3	4225	414	23
29.4	1.46	3.4	4077	285	85
3.8	0.15	0.4	1451	93	41
5	5	5	5	5	5
53	1.4	2.2	3650	296	32
72	4.0	2.9	63	4	12
38	7.5	4.6	6705	750	284
120	3.5	3.6	6137	409	42
26	3.5	2.8	2815	241	20
53	3.2	3.7	5460	196	38
10	4.4	7.4	3100	325	39
29	3.7	2.7	5805	557	38
50.0	3.9	3.7	4217	347	63
12.0	0.60	0.6	788	81	32
8	8	8	8	8	8
42	3.0	3.6	4163	323	71.5
7.9	0.5	0.4	702	59	24
13	13	13	13	13	13

Appendix VI. Physical and hormonal characteristics of fetuses at 180 days gestation.

Sex of fetus	Cow (or fetal) number	Age	Body weight	Crown-Rump length	Paired adrenal weight	Paired testicular or ovarian weight	Seminal vesicular or uterine weight
		(days)	(kg)	(cm)	(mg)	(mg)	(mg)
Male	540	184	4.54	45.7	545	952	188
	542	179	6.30	50.8	856	1149	335
	554	179	5.92	55.9	827	1023	333
	555	179	7.14	58.4	682	1780	460
	556	187	6.68	53.3	608	1392	642
	558	178	6.14	53.3	804	1539	646
	559	178	7.90	54.6	797	1856	1002
	Mean	180.6	6.37	53.1	731	1384	515
	S.E.	1.3	0.40	1.5	45	136	103
	n	7	7	7	7	7	7
Female	541	183	5.91	50.8	750	197	1619
	546	178	7.23	55.9	969	174	1977
	547	177	7.44	58.4	834	551	2426
	551	178	6.15	57.2	737	148	1491
	557	185	6.02	55.9	585	416	2519
	567	180	6.20	52.1	760	234	1817
	Mean	180.2	6.49	55.1	773	287	1975
	S.E.	1.3	0.27	1.2	52	66	172
	n	6	6	6	6	6	6
Mean		180.4	6.43	54.0	750	--	--
S.E.		0.9	0.24	1.0	33	--	--
n		13	13	13	13	--	--

Appendix VI. Con't.

Umbilical blood serum hormones						Anterior pituitary hormones					
GH		LH		Prolactin		Content			Net in vitro synthesis /mg/72 hrs		
Artery	Vein	Artery	Vein	Artery	Vein	GH	LH	Prolactin	GH	LH	Prolactin
(ng/ml)									(µg)	(ng)	(µg)
84	85	0.7	0.7	30	28	--	--	--	--	--	--
66	58	1.0	1.0	70	30	--	--	--	--	--	--
115	120	1.0	1.0	26	25	6939	581	704	3.8	551	17.1
54	50	0.7	0.7	60	55	12807	253	2917	-2.5	859	16.1
53	47	3.2	3.2	11	9	5050	474	275	-1.1	258	5.2
32	31	0.8	0.9	21	16	5797	220	207	8.4	406	11.8
50	55	0.5	0.5	19	16	14680	539	411	-7.6	1203	2.0
65	64	1.12	1.14	34	26	8911	413	903	1.2	655	10.4
10	11	0.35	0.34	8.4	5.6	1896	74	510	2.6	169	3.0
7	7	7	7	7	7	5	5	5	5	5	5
66	66	1.8	1.7	138	120	--	--	--	--	--	--
109	122	1.5	1.7	68	62	11082	372	1836	93.4	245	134.6
50	47	0.6	0.7	21	46	8573	545	1891	-1.1	-194	24.9
54	53	0.7	0.7	83	81	11783	324	1881	-5.0	463	14.9
46	45	2.5	2.3	21	13	8440	582	1109	-1.2	-25	11.6
70	54	1.7	1.7	18	19	4974	850	268	-0.6	570	1.9
66	65	1.47	1.47	58	57	8970	535	1397	17.1	212	37.5
9	12	0.29	0.26	20	16	1200	93	318	19.1	144	24.0
6	6	6	6	6	6	5	5	5	5	5	5
65	64	1.28	1.29	45	40	8940	474	1150	8.7	434	24.0
7	8	0.23	0.22	10	9	1058	60	295	9.5	128	12.5
13	13	13	13	13	13	10	10	10	10	10	10

Appendix VII. Physical and hormonal characteristics of fetuses at 260 days gestation.

Sex of fetus	Cow (or fetal) number	Age	Body weight	Crown-Rump length	Paired adrenal weight	Paired testicular or ovarian weight	Seminal vesicular or uterine weight
		(days)	(kg)	(cm)	(mg)	(mg)	(mg)
Male	515	248	38.9	92.7	1788	6488	1933
	524	253	21.4	78.7	1403	3124	1026
	539	242	30.2	91.4	1829	3304	1053
	549	264	33.2	91.4	1953	5429	923
	562	265	14.1	67.3	1869	2968	616
	566	264	29.5	90.2	1885	3502	1863
	Mean	256	27.9	85.3	1788	4136	1236
	S.E.	4.0	3.6	4.2	80	597	219
	n	6	6	6	6	6	6
Female	525	261	19.3	73.7	1394	864	2863
	550	264	18.6	78.7	1095	311	3711
	560	265	30.0	88.9	1763	1011	6600
	563	265	37.7	99.1	2493	1627	7133
	564	262	20.5	76.2	1496	1321	4273
	Mean	263	25.2	83.3	1648	1027	4916
	S.E.	0.1	3.7	4.7	236	222	832
	n	5	5	5	5	5	5
Mean		259	26.7	84.4	1724	--	--
S.E.		2.4	2.5	3.0	111	--	--
n		11	11	11	11	--	--

Appendix VII. Con't.

Umbilical blood serum hormones						Anterior pituitary hormones					
GH		LH		Prolactin		Content			Net in vitro synthesis /mg/72 hrs		
Artery	Vein	Artery	Vein	Artery	Vein	GH	LH	Prolactin	GH	LH	Prolactin
(ng/ml)									(µg)	(ng)	(µg)
137	129	1.2	1.2	134	179	9803	482	1407	-4.8	277	10.6
117	103	1.6	1.6	114	86	7418	848	3743	-5.8	1273	-1.2
106	115	0.7	0.7	56	66	3788	254	2821	1.8	331	23.6
88	89	0.7	0.7	32	20	30614	701	4899	-2.2	167	11.8
138	153	0.4	0.5	6	6	2732	393	1610	4.7	30	8.4
68	65	0.6	0.6	14	6	18300	974	1077	10.3	-439	19.0
109	109	0.87	0.88	59	61	12109	609	2593	0.68	273	12.1
11	13	0.18	0.17	22	27	4341	114	615	2.5	230	3.5
6	6	6	6	6	6	6	6	6	6	6	6
70	65	0.6	0.7	93	87	15301	670	3417	-9.1	-105	65.6
70	64	1.9	1.9	114	79	11209	328	2710	12.8	641	57.6
119	121	0.5	0.4	24	20	18626	192	1743	15.7	354	14.7
51	58	0.5	0.6	24	28	35580	728	2511	-2.8	-157	22.9
157	192	0.7	0.5	87	70	45609	312	1660	-2.1	-27	22.0
93	100	0.84	0.82	68	57	25265	446	2408	2.8	141	36.5
20	26	0.26	0.27	19	14	6562	106	325	4.8	153	10
5	5	5	5	5	5	5	5	5	5	5	5
102	105	0.85	0.85	63	59	18089	535	2508	1.7	213	23.2
10	13	0.14	0.15	14	15	4154	79	351	2.5	138	6.2
11	11	11	11	11	11	11	11	11	11	11	11

Appendix VIII. Hormone content of pituitary explants and pituitary incubation media.

Age	Sex of fetus	Cow (or fetal) number	Weight of explant	Pituitary explants			Incubation media		
				GH	LH	Prolactin	GH	LH	Prolactin
			(mg)	(ug/mg)	(ng/mg)	(μg/mg)	(ug)[a]	(ng)[a]	(g)[a]
180	Male	554	1.9	1.5	135	9.7	9.2	997	8.1
		555	2.5	4.7	1042	2.5	5.6	70	16.5
		556	3.2	0.8	308	0.9	3.1	424	4.6
		558	4.3	1.9	129	1.2	12.3	497	10.8
		559	4.6	1.6	925	0.4	5.5	817	2.1
		Mean		2.1	508	2.9	7.1	561	8.4
		S. E.		0.7	197	1.7	1.6	161	2.5
		n		5	5	5	5	5	5
	Female	546	1.3	6.8	5	0.8	97.7	612	135.6
		547	3.1	3.6	110	2.2	3.8	241	24.6
		551	(2.7)[b]	1.4	245	1.9	5.4	542	14.9
		557	3.2	1.0	187	0.9	6.3	370	11.8
		567	2.7	1.2	514	0.2	3.2	906	2.0
		Mean		2.8	212	1.2	23.3	534	37.8
		S. E.		1.1	85	0.4	18.6	113	24.7
		n		5	5	5	5	5	5
260	Male	515	1.3	0.9	243	0.4	4.1	516	11.6
		524	1.8	1.2	649	0.2	0.5	1472	2.6
		539	3.5	0.4	76	0.3	5.2	509	26.1
		549	(2.7)[b]	3.2	246	1.6	5.2	622	15.1
		562	3.8	1.0	228	2.8	6.5	195	7.2
		566	2.7	9.6	230	4.4	19.0	305	15.6
		Mean		2.7	279	1.6	6.8	603	13.0
		S. E.		1.4	79	0.7	2.6	185	3.3
		N		6	6	6	6	6	6
	Female	525	2.3	0.4	90	3.8	5.8	475	65.2
		550	(2.7)[b]	15.6	465	5.9	8.3	504	54.4
		560	3.5	14.1	249	1.9	20.1	297	14.6
		563	3.2	8.0	262	4.3	24.7	309	21.1
		564	6.0	15.6	208	2.6	8.6	77	20.9
		Mean		10.7	255	3.7	13.5	332	35.2
		S. E.		2.9	61	0.7	3.7	76	10.2
		n		5	5	5	5	5	5

[a] Per mg pituitary per 72 hr incubation.

[b] Average of all slices used because weights of slices lost.