INVESTIGATIONS INTO
CONGENITAL HYPOTHYROIDISM
OF FOALS

A Thesis

Submitted to the College of Graduate Studies and Research

in Partial Fulfilment of the Requirements

for the Degree of

Doctor of Philosophy

in the

Department of Veterinary Pathology

Western College of Veterinary Medicine

University of Saskatchewan

Saskatoon

By

Andrew Lyndon Allen

October 1996

UNIVERSITY OF SASKATCHEWAN

College of Graduate Studies and Research

SUMMARY OF DISSERTATION

Submitted in partial fulfillment
of the requirements for the

DEGREE OF DOCTOR OF PHILOSOPHY

by

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October 9, 1996

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Investigations into Congenital Hypothyroidism of Foals

A naturally occurring disease involving hyperplasia of the thyroid gland and a consistent pattern of musculoskeletal deformities of newborn foals in western Canada was first described in 1981. This disease was an important cause of foal mortality and, therefore, reproductive loss throughout western Canada during the 1980s and has since been recognized in western Ontario and the northwestern United States. A series of investigations were conducted to describe, characterize, and attempt to determine the pathogenesis and cause of this syndrome.

Affected foals were typically born after a long gestation ($\bar{x}$ = 360 days, range = 340 to 400 days), were diagnosed as hypothyroid based on a poor response to the administration of thyroid-stimulating hormone, and had various musculoskeletal lesions of which mandibular prognathism, flexural deformities and rupture of tendons of the limbs, and incomplete ossification of the carpal and tarsal bones were present most commonly. In spite of the normal to long gestation, foals had signs of immaturity, were usually weak and unable to stand, became septic, and died or were euthanatised.

Similar histories, clinical findings, and lesions were present in surgically created hypothyroid foals that were thyroidectomized in utero at about 210 days gestation. These findings supported the conclusion that foals which naturally developed these lesions were also hypothyroid in utero and that all the lesions present in affected foals were the result of the hypothyroidism and not of an underlying concurrent disease process.

A case-control study was conducted to identify risk factors for naturally occurring congenital hypothyroidism. Information from congenitally hypothyroid foals concerning foal and dam signalment, farm environment, and dam management was compared with that from normal foals. Pregnant mares fed greenfeed, not supplemented with mineral, that left their "home farm"

during gestation, or grazed irrigated pasture, had a 13.1 ($P = 0.0068$), 5.6 ($P = 0.0472$), 4.3 ($P = 0.0076$) and approximately 15.3 ($P = 0.0245$) times greater odds, respectively, of producing a congenitally hypothyroid foal than mares not exposed to these factors.

Greenfeed often contains high levels of nitrate (NO_3^-) which is known to impair thyroid gland function. In light of this, forage samples from participating farms were analysed for nitrate levels. The odds of one or more congenitally hypothyroid foal being born on a farm feeding forage with at least a trace of nitrate was 8.0 times greater ($P = 0.0873$) than the odds of the disease occurring a farm that fed forage free of nitrate. Further, the odds of a mare producing an affected foal when fed forage containing at least a trace of nitrate was 5.9 times greater ($P = 0.0007$) than a mare fed nitrate-free forage. This study suggests that congenital hypothyroidism in foals may result from diets containing nitrate or low in iodine being fed to pregnant mares.

These results need to be confirmed through further field investigations and controlled experiments. However, if they are accurate, there is cause for concern in that other livestock raised those areas where congenitally hypothyroid foals occur may be exposed to the same dietary risk factors and may suffer similar disease.

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1987	Doctor of Veterinary Medicine, University of Saskatchewan
1992	Master of Veterinary Science, University of Saskatchewan

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ABSTRACT

A naturally occurring disease involving hyperplasia of the thyroid gland and a consistent pattern of musculoskeletal deformities of newborn foals in western Canada was first described in 1981. This disease was an important cause of foal mortality and, therefore, reproductive loss throughout western Canada during the 1980s and has since been recognized in western Ontario and the northwestern United States. A series of investigations were conducted to describe, characterize, and attempt to determine the pathogenesis and cause of this syndrome.

Affected foals were typically born after a long gestation ($\bar{x}$ = 360 days, range = 340 to 400 days), were diagnosed as hypothyroid based on a poor response to the administration of thyroid-stimulating hormone, and had various musculoskeletal lesions of which mandibular prognathism, flexural deformities and rupture of tendons of the limbs, and incomplete ossification of the carpal and tarsal bones were present most commonly. In spite of the normal to long gestation, foals had signs of immaturity, were usually weak and unable to stand, became septic, and died or were euthanatised.

Similar histories, clinical findings, and lesions were present in surgically created hypothyroid foals that were thyroidectomized in utero at about 210 days gestation. These findings supported the conclusion that foals which naturally developed these lesions were also hypothyroid in utero and that all the lesions present in affected foals were the result of the hypothyroidism and not of an underlying concurrent disease process.

A case-control study was conducted to identify risk factors for naturally occurring congenital hypothyroidism. Information from congenitally hypothyroid foals concerning foal and dam signalment, farm environment, and dam management was compared with that from normal foals. Pregnant mares fed greenfeed, not supplemented with mineral, that left their "home farm" during gestation, or grazed irrigated pasture, had a 13.1 ($P = 0.0068$), 5.6 ($P = 0.0472$), 4.3 ($P = 0.0076$) and approximately 15.3 ($P = 0.0245$) times greater odds, respectively, of producing a congenitally hypothyroid foal than mares not exposed to these factors.

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These results need to be confirmed through further field investigations and controlled experiments. However, if they are accurate, there is cause for concern that other livestock raised in areas where congenitally hypothyroid foals occur may be exposed to the same dietary risk factors and may suffer similar disease.

ACKNOWLEDGEMENTS

The work reported in this thesis would not have been possible without the financial support of the following granting agencies:

Alberta Agricultural Research Institute
Alberta Racing Commission Development Fund
Natural Sciences and Engineering Research Council of Canada
Saskatchewan Agriculture Development Fund
Western College of Veterinary Medicine Equine Health Research Fund.

The contribution of resources, both material and human, by the Department of Veterinary Pathology and the donations of radiographic film by Dupont Canada and Kodak Canada were integral to this research and are hereby acknowledged.

Dr. Allen was funded by an Interprovincial Graduate Student Scholarship during his Doctor of Philosophy program. A Fellowship from the Western College of Veterinary Medicine Equine Health Research Fund to support Dr. Allen during earlier graduate training is also recognized.

Many people contributed to this research including the faculty, professional associates, graduate students and support staff of the Department of Veterinary Pathology; the members of the Diagnostic Laboratory and Diagnostic Clinical Pathology; Drs. Nigel Caulkett, Tanya Duke, Christine Egger, and the technicians of the veterinary anesthesia service; the support staff of the Large Animal Clinic; employees of the Animal Care Unit; the veterinary practitioners in Alberta and other areas of western Canada; and the many horse owners and farm managers who, through their cooperation, made these studies possible.

Dr. Mike Scott, Dr. Claire Card, Dr. Shawn Haas, Dr. Antonio Cruz, Dr. Christine Theoret, Dr. John Pharr, Dr. Rachel St-Vincent, Dr. Nadia Cymbaluk, and Ms. Sue Cook gave generously of their time and expertise; their contributions were vital to this research.

A special expression of gratitude is owed to Dr. Cecil Doige for his mentorship; to Dr. Peter Fretz who was the person most responsible for the conduct of this research; to Dr. Hugh Townsend for his instruction and invaluable contributions in the area of scientific investigation; and to Dr. Craig Riddell, Dr. Ted Leighton, and Dr. Dorothy Middleton for their leadership, patience, and encouragement.

DEDICATION

This thesis is dedicated to

Dr. Cecil E. Doige

- I hope he would have been proud of this work -

and to my parents,

Frank and Eileen Allen

- they deserved the opportunity to be proud of me.

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LIST OF ABBREVIATIONS, SYMBOLS AND ACRONYMS USED IN THIS THESIS

Standard Abbreviations and Abbreviations and Symbols Used in Medicine

A:	a variable; used in this thesis to represent the *y*-intercept of a straight line
B:	a variable; used in this thesis to represent the slope of a straight line
b.i.d.:	*bis in die*; twice a day
°C:	degrees Celsius
cm:	centi ($\times 10^{-2}$) meter(s)
CV:	coefficient(s) of variation
et al.:	*et alia*; and others
g:	gram(s)
g:	standard gravity (9.80616 meters per second squared)
g/kg:	gram(s) per kilogram
i.e.:	*id est*; that is
IU:	international unit(s)
IU/kg:	international unit(s) per kilogram
kg:	kilo ($\times 10^{3}$) gram(s)
mg:	milli ($\times 10^{-3}$) gram(s)
mm:	milli ($\times 10^{-3}$) meter(s)
nmol/L:	nano ($\times 10^{-9}$) mole(s) per liter
ng/ml:	nano ($\times 10^{-9}$) gram(s) per milli ($\times 10^{-3}$) liter(s)
P:	*P*-value or probability

r:	correlation coefficient; a measure of the strength and direction of a linear association between two variables
s:	sample standard deviation
SI:	Système International d'Unitès; International System of Units
T_3:	triiodothyronine
T_4:	thyroxine
TRH:	thyrotropin-releasing hormone
TSH:	thyroid-stimulating hormone (also known as thyrotropin)
μg/kg:	micro ($\times 10^{-6}$) gram(s) per kilogram
μm:	micro ($\times 10^{-6}$) meter(s)
$\times$:	multiplied by
x:	a variable; used in this thesis to represent a hormone concentration
$\bar{x}$:	sample mean
y:	a variable; used in this thesis to represent an amount of polarization in the fluorescence polarization immunoassay
>:	greater than
<:	less than
$\leq$:	less than or equal to
-	minus
#:	number
%:	percent or of each hundred
$\pm$	plus and minus

Acronyms Used in this Thesis

CB:	competitive binding
CDET:	tendon of the common digital extensor muscle(s)
CF:	conversion factor
CHD:	congenital hypothyroidism and dysmaturity, or congenitally hypothyroid and dysmature
CI:	confidence interval
cOR:	crude odds ratio
ELISA:	enzyme-linked immunosorbent assay
FPIA:	fluorescence polarization immunoassay
na:	not appropriate
NO_2^-:	nitrite
NO_3^-:	nitrate
nr:	not recorded
OR:	odds ratio
ORmh:	Mantel-Haenszel summary odds ratio
RIA:	radioimmunoassay
sOR:	stratum specific odds ratio
TH-MSD:	thyroid gland hyperplasia and musculoskeletal deformities
WCVM:	Western College of Veterinary Medicine

LIST OF COMMERCIAL PRODUCTS USED AND REFERRED TO IN THIS THESIS

Product Description	*Brand name, Manufacturer and Address*
altrenogest	Regu-mate gel, Hoechst Canada, Regina, Saskatchewan
ampicillin	Penbritin, Ayerst Laboratories, Montreal, Quebec
bovine TSH[a] (thyroid-stimulating hormone)	Thytropar, Rorer Canada, Bramalea, Ontario
bovine TSH[b] (thyroid-stimulating hormone)	thyrotropic hormone, Sigma Chemicals, St. Louis, Missouri
butorphanol tartrate	Torbugesic, Ayerst Laboratories, Saint Laurent, Quebec
4% chlorhexidine gluconate	Hibitane skin cleanser, Ayerst Laboratories, Montreal, Quebec
clenbuterol hydrochloride	Ventipulmin solution or Ventipulmin syrup, Boehringer Ingelheim (Canada), Burlington, Ontario
computer software	InStat2, Copyright 1993: GraphPad Software, San Diego, California
computer software	Prism, Copyright 1994-1995: GraphPad Software, San Diego, California
computer software	Statistical Analysis System, Copyright 1989: SAS Institute, Cary, North Carolina

dobutamine hydrochloride.....Dobutrex solution, Eli Lilly Canada, Toronto, Ontario

equine encephalitis vaccine with tetanus toxoid.....Double-ET, Solvay Animal Health, Kitchener, Ontario

equine herpesvirus (strains 1p and 1b) vaccine.....Pneumabort K+1b, Fort Dodge Laboratories, Fort Dodge, Iowa

equine influenza and herpesvirus vaccine.....Fluvac, Fort Dodge Laboratories, Fort Dodge, Iowa

flunixin meglamine.....Banamine solution, Schering-Plough Animal Health, Pointe-Claire, Quebec

FPIA (fluorescence polarization immunoassay).....Abbott TDx System; Abbott Laboratories, North Chicago, Illinois

glyceryl guaiacolate.....guaifenesin or guaiacol glyceryl ether, Sigma Chemicals, St. Louis, Missouri

granular vitamin and mineral supplement.....Weatherguard #21, Prairie Micro-tech, Regina, Saskatchewan

halothane.....halothane, Halocarbon Laboratories, River Edge, New Jersey

70% isopropyl alcohol.....isopropyl rubbing alcohol, Medibiotics, Mississauga, Ontario

ivermectin for cattle.....Ivomec, Merck AgVet, Kirkland, Quebec

ivermectin for horses.....Eqvalan liquid or Eqvalan paste, Merck AgVet, Pointe Claire-Dorval, Quebec

ketamine hydrochloride.....Ketalean, MTC Pharmaceuticals, Cambridge, Ontario

monofilament polydioxanone suture.....PDS II, Ethicon, Somerville, New Jersey

oxytocin.....Oxytocin, Rogar/STB, London, Ontario

procaine penicillin G.....Ethacilin, Rogar/STB, London, Ontario

rabies vaccine.....Rabguard-TC, SmithKline Beecham Animal Health, West Chester, Pennsylvania

sodium chloride salt blocks with added iodine and cobalt.....Cobalt Iodized Stock Salt, Canadian Salt Co., Pointe-Claire, Quebec

sodium penicillin G.....penicillin g sodium, Novopharm, Scarborough, Ontario

sodium pentobarbital.....Euthanyl Forte, MTC Pharmaceuticals, Cambridge, Ontario

stainless steel staples.....Appose ULC, Davis & Geck, Wayne, New Jersey

sterile, evacuated, 10 ml, glass tubes.....Vacutainer Systems, Becton Dickinson, Rutherford, New Jersey

trimethoprim and sulfamethoxazole.....Nu-cotrimox, Nu-Pharm, Scarborough, Ontario

trimethoprim and sulphadiazine.....Tribrissen 48% injection, Janssen Pharmaceutica, Mississauga, Ontario

trimethoprim and sulphadoxine.....Borgal, Hoechst Canada, Regina, Saskatchewan

xylazine hydrochloride.....Rompun, Bayer, Etobicoke, Ontario

1.0 GENERAL INTRODUCTION AND REVIEW OF LITERATURE

In the late 1970s and early 1980s, veterinarians at the Western College of Veterinary Medicne (WCVM) were engaged in landmark studies of angular limb deformities in foals (Turner and Fretz 1977; Fretz et al. 1978; Fretz 1980; McLaughlin et al. 1981; Pharr and Fretz 1981; Fretz and Donnecker 1983; Fretz and McIlwraith 1983; Fretz and Cymbaluk 1984). Among the foals examined during this period were several that were atypical of the rest, but with similarities to each other. In 1981, McLaughlin and Doige described the lesions found postmortem in 7 of these foals. Multiple musculoskeletal abnormalities were common and included mandibular prognathism, forelimb contracture (i.e., flexural deformity), rupture of the common digital extensor tendons, and severely retarded ossification of the carpal and tarsal bones. All of the foals also had grossly normal appearing, but microscopically hyperplastic, thyroid glands leading to speculation that these foals may have had abnormal thyroid function during the perinatal period.

Support for this theory was provided by a subsequent study by McLaughlin and Doige (1982) when they demonstrated that surgical thyroidectomy of 1 day old foals resulted in a decrease in the rate of ossification of the carpal and tarsal bones. Then, in 1986, McLaughlin et al. reported that they had measured the triiodothyronine (T_3) and

thyroxine (T_4) levels in an additional 14 foals with various combinations of hyperplastic thyroid glands, angular limb deformities, forelimb contracture, ruptured common digital extensor tendons, mandibular prognathism, and incompletely ossified carpal or tarsal bones. Ten of the 14 foals had low levels of T_3, T_4, or both compared to thyroid hormone levels in clinically healthy foals from the same geographic area. These latter levels were in agreement with published values of foals of similar age (Irvine and Evans 1975; Blackmore et al. 1978; Chen and Riley 1981). In addition, the response of 2 foals to the intramuscular administration of 15 IU of thyroid-stimulating hormone (TSH) was monitored and considered poor.

Finally, Allen et al. (1994) reported on the results of a retrospective study of the records of the 8 veterinary diagnostic laboratories in western Canada. They confirmed that a syndrome of thyroid gland hyperplasia and a specific set of musculoskeletal deformities (TH-MSD) existed and had occurred consistently over many years and in several regions of western Canada. Typically foals with the TH-MSD syndrome had a long gestation ($\bar{x}$ = 360 days), were born weak and unable to stand, were often unusually small or unusually large, and had several signs of immaturity such as lax tendons and joints, pliable ears, and a short hair coat. Consistent musculoskeletal anomalies included mandibular prognathism, flexural and angular deformities of the forelimbs and hind limbs, and poorly ossified carpal and tarsal bones.

Allen et al. (1994) also found that there was no sex or breed predilection among foals with the TH-MSD syndrome. Since neither the sex nor breed of a foal appeared to increased its chance of developing the disease a heritable condition was not suspected

(Nicholas 1996). At the same time, Allen et al. (1994) noticed that almost one-third of all the TH-MSD foals were born on farms that had other affected foals, and suspected that an environmental determinant of the disease, such as a dietary deficiency, toxic substance, or infectious agent, may be responsible.

The perception that the incidence of this disease was increasing (Kreplin and Allen 1991), combined with a pledge of increased cooperation and resources on the part of individual horse breeders as well as breed associations, provided the means, motivation and momentum to renew and intensify investigations into the TH-MSD syndrome of foals.

1.1 Hypothyroidism in the Horse

1.1.1 Maturing and mature horses

Despite earlier (Schlotthauer 1931; Abbott and Prendergast 1934; Dimock et al. 1944) and more recent (Dalefield and Palmer 1994) suggestions that morphological lesions of the thyroid gland of horses are common, investigators of thyroid function in the horse (Beech and Garcia 1991; Messer 1993; Sojka et al. 1993; Messer 1994; Messer et al. 1995a, 1995b; Sojka and Levy 1995; Sojka 1996) agree that confirmed cases of abnormal thyroid function in horses, other than neonates, are rare (Held et al. 1985). However, these same reviews of thyroid gland disease in horses frequently refer to previous suggestions that hypothyroidism is associated with laminitis (Britton 1959), anhidrosis (Smythe 1963; Correa and Calderin 1966), and poor athletic performance combined with rhabdomyolysis during exercise (Waldron-Mease 1979).

Although these associations have not been ruled out with definitive studies, neither have they been supported by published literature since they were first proposed in the late 1950s, early 1960s, and late 1970s, respectively.

Hypothyroidism was created in horses and ponies, which were between 1 month and 3 years of age, by surgical thyroidectomy by Lowe and others at Cornell University (Lowe and Kallfelz 1970; Lowe et al. 1974, 1975, 1987). The horses in those studies became docile and lethargic; were sensitive to cold; failed to grow and mature; developed a dull, coarse hair coat that was late to shed and became sparse; exhibited thickened, wrinkled skin, particularly over the face; developed edema of the rear limbs; and had lower rectal temperatures, lower hematocrits, and higher blood cholesterol levels compared to control animals.

Two case reports which are most consistent with and convincing of naturally occurring hypothyroidism in horses (Stanley and Hillidge 1982; Hillyer and Taylor 1992) describe abnormalities limited to or primarily concerned with the hair coat and skin. The histories and clinical findings of these 2 horses were similar to each other and to those of the experimentally created hypothyroid horses.

1.1.2 Congenital hypothyroidism in foals

Investigators, independent of the group at the WCVM, have described the clinical and postmortem findings of foals presumed to be congenitally hypothyroid. Interestingly, these descriptions were varied and often vague. It appears that part of the imprecision in characterizing congenital hypothyroidism in foals is the confusion

between morphological abnormalities of the thyroid gland and abnormalities in thyroid function.

One of the earliest reports of congenital thyroid gland disease of foals is that of Rodenwold and Simms (1934) who described experiences at the Oregon State College between 1910 and 1930. Foals born to mares fed a diet suspected to be deficient in iodine were described as being full term, but weak and often unable to stand at birth. Several of these foals had "a pronounced enlargement of the thyroid gland", flexural deformities of the forelimbs, and developed enlarged joints. Mortality was high among affected foals. Another report of suspected iodine deficiency in pregnant mares described premature delivery of weak fetuses and foals with flaccid bodies and goitre (Yong and Griffin 1992).

Baker et al. (1983) described a case of congenital colloidal goitre in a foal which also exhibited hyperextension of the lower limbs and difficulty nursing. The authors believed the foal had normal thyroid function. A combination of iodine deficiency and ingestion of a goitrogen (soya meal) by the dam was considered to be the cause of the problems in the foal.

There are several published reports of mares, receiving a diet believed to contain excessive iodine, giving birth to goitrous foals. Baker and Lindsey (1968) described several foals from 3 different farms as having "striking thyroid enlargement" as a result of follicles being distended with colloid. The foals were also weak and unable to stand because of contracted front legs. Mares producing goitrous foals received 48 to 432 mg

of iodine daily. In contrast, mares on a 4th farm, where no goitre was observed, received no more than 7.0 mg of iodine daily.

Other mares fed a diet with similarly high levels of iodine (160 to 400 mg daily) produced foals with "hyperplastic colloid goitre" that were otherwise normal (Sippel 1968). A description of mares which ingested lower levels of iodine (83 mg daily) than the previous 2 reports, but levels that were still considered excessive, produced weak foals which were unable to stand, had enlarged hyperplastic thyroid glands, and died soon after birth (Drew et al. 1975). Driscoll et al. (1978) identified 2 foals with enlarged thyroid glands and Miyazawa et al. (1978) described 3 foals with enlarged, firm thyroid glands. The dams of these foals received 35 mg and 30 mg of iodine per day, respectively. The foals were, otherwise, normal and because Miyazawa et al. (1978) believed the foals they examined had normal thyroid function, they described the thyroid gland lesion as nodular goiter.

Conway and Cosgrove (1980) found that mares fed an unspecified high level of iodine delivered foals which were "backward in condition", retarded in their skeletal development, and had "parenchymatous goitre". In 1987, Silva et al. also reported on the effects of feeding pregnant mares a diet containing excess iodine. Of 39 mares at risk, 17 aborted goitrous fetuses, 1 mare produced a stillborn foal which had a "striking goitre", and 3 mares gave birth to weak foals which "showed goitre" and were killed. Two of the foals which had been killed also had "considerable alterations in the skeleton" including osteopetrosis of the cannon bones. The circulating concentrations of both T_3 and T_4 in the foals whose dams consumed the iodine excessive diet were

higher than the concentrations of normal foals used for comparison. However, no opinion about thyroid function in these foals was offered.

Durham (1995) found 2 of 6 mares, being fed 26 mg of iodine per day during the last 3 or 4 months of gestation, produced congenitally goitrous foals. Both foals were born without complications and were, in all other respects, clinically normal.

Finally, in 1977, Irvine and Evans described 3 foals that were weak, uncoordinated, and failed to stand; were sluggish and lacked appropriate behavior; were goitrous; and were considered hypothyroid based on low levels of T_3, total T_4 and free T_4. The cause of the goitre and low thyroid hormone levels was not identified.

1.2 Thesis

From the above discussions and ones that will follow, it is apparent that, while naturally occurring hypothyroidism in the horse has been of concern to veterinarians for several decades, little is known about the acquired form of this condition in mature animals or the congenital form of the condition in neonates. Confusion surrounds such fundamental issues as the relationship between morphological abnormalities and functional abnormalities of the thyroid gland, the manifestations of hypothyroidism which arouse suspicion of the disease, and the appropriate tests used to confirm or exclude the diagnosis.

This thesis will focus on investigations of congenital hypothyroidism in foals. The motivation for these investigations was to increase the understanding of the disease currently referred to as TH-MSD (Allen et al. 1994) which has been described with

regularity in western Canada. At the same time, there is an increasing amount of evidence to indicate that TH-MSD foals are born in several regions of North America. In 1975, 2 veterinary practitioners in the Minnesota-Wisconsin area reported on 10 foals with bilateral rupture of the common digital extensor tendons (Myers and Gordon 1975). Some of these foals also featured flexural deformities of the front legs, hind legs, or both; mandibular prognathism; underdeveloped pectoral muscles; and retarded skeletal development. More recently, there has been a published report describing over 20 TH-MSD foals diagnosed in the northwestern United States during the previous 3 years (Hines et al. 1996). These accounts serve to support the numerous personal communications this author has had with horse owners and veterinarians reporting TH-MSD foals from western Ontario, the northwestern United States, and Australia.

The similarities between presumed congenital hypothyroidism in foals and TH-MSD are obvious and yet the understanding of either disease is sufficiently poor that it is currently impossible to know if congenital hypothyroidism and TH-MSD are the same disease or different. Regardless of the relationship between congenital hypothyroidism and TH-MSD, the cause of latter remains unknown. The TH-MSD syndrome occurs commonly in western Canada and is an important cause of foal mortality. It is incumbent on those at the WCVM to investigate the TH-MSD syndrome of foals with the objective of reducing losses through prevention or the successful treatment of those affected.

1.2.1 General objectives

The collective objective of the investigations in this thesis was to increase the understanding of the TH-MSD syndrome of foals. While this is a vague expression of one's intentions, this general approach might be anticipated and considered prudent when initiating the investigation of a disease with which there has been limited experience, which has been infrequently described, and which has been unpredictable in its occurrence. As would be the case with any poorly understood clinical entity, increasing the understanding of the TH-MSD syndrome meant being able to recognize affected foals when they occurred while being able to exclude other foals suffering with a similarly appearing disease which could be confused with the TH-MSD syndrome. This required employing the case definition provided by Doige and his coworkers (McLaughlin and Doige 1981; McLaughlin et al. 1986; Allen et al. 1994) and refining the definition as more was learned about the disease (Allen et al. 1993). With accurate case recognition it would be possible to describe the chronologic and geographic pattern of the disease, and with experience, predict its occurrence. With the ability to predict the occurrence of affected foals it would be possible to conduct studies concerning risk factors for the TH-MSD syndrome. In turn, identifying risk factors for the disease would eventually allow for speculation as to the pathogenesis and ultimately, through hypothesis testing, identification of the cause. As was stated above, knowledge about the cause and pathogenesis of the TH-MSD syndrome would provide the means to prevent further disease and direct treatment of affected foals at the cause or at

intervention in the pathogenesis, as opposed to the simple and nonspecific relief of symptoms.

1.2.2 Specific objectives

This thesis research had the following specific objectives:

1) To develop a reliable thyroid function test for neonatal foals using the resources available at the WCVM.

2) To determine if foals with the TH-MSD syndrome had normal thyroid function.

3) To describe further the signalment, history, clinical signs and postmortem lesions of TH-MSD foals.

4) To investigate the pathogenesis of the TH-MSD syndrome using a model of in utero hypothyroidism in foals.

5) To investigate the risk factors for naturally occurring cases of TH-MSD in foals.

6) To provide testable hypotheses about the cause of naturally occurring cases of TH-MSD in foals.

1.3 Use of Animals in this Thesis Research

All procedures described in this thesis involving the use of animals received approval from the University Committee on Animal Care and Supply at the University of Saskatchewan, and were conducted in accordance with the guidelines established by the Canadian Council on Animal Care.

2.0 THYROID FUNCTION TESTING IN HORSES[1]

2.1 Introduction

An increased understanding of normal thyroid physiology combined with continuous advances in technologies has changed the way veterinarians have tested thyroid function in animals (Hightower et al.1969; Bustad and Fuller 1970; Hightower et al. 1971). Current recommendations for assessing thyroid function in horses involve the measurement of the circulating thyroid hormones triiodothyronine (T_3) and thyroxine (T_4), preferably in conjunction with thyroid-stimulating hormone (TSH) or thyrotropin-releasing hormone (TRH) administration (Beech 1987; Messer 1993, 1994; Sojka and Levy 1995; Sojka 1996). The most common and reliable methods of measuring T_3 and T_4 are competitive binding (CB) and radioimmunoassay (RIA) (Beech 1987). A less common but equally reliable method involves a fluorescence polarization immunoassay (FPIA) (Spring et al. 1983; Symons and Vining 1985). This assay was available in the Endocrine Laboratory, Department of Veterinary Physiological Sciences, Western College of Veterinary Medicine (WCVM).

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[1] Portions of this chapter have been published (see Allen et al. 1995) or are "in press" (see Allen et al. 1996b).

2.1.1 Overview

It has been suggested that thyroid function can be assessed with a single measurement of circulating thyroid hormone concentrations (Beech 1987; Nachreiner and Hyland 1993). However, several investigators have found that a variety of factors may influence T_3 and T_4 concentrations and warn against using baseline levels as the means of differentiating euthyroid and hypothyroid horses (Sojka 1993; Messer 1993, 1994; Sojka and Levy 1995; Sojka 1996). Besides the inherent variability expected between horses, there is evidence that the levels of thyroid hormones may vary within the same horse under different circumstances.

2.1.2 Triiodothyronine and thyroxine concentrations in normal horses

Published papers reporting thyroid hormone concentrations in healthy horses were used to construct tables which crudely summarized the findings of several investigators, working at different times, in a variety of locations, and under different conditions. The results are summarized in Appendices A, B, C, and D. Triiodothyronine and thyroxine were considered independently, and separate tables were constructed for foals from birth to about 1 year of age, and for horses from about 1 year of age and older. In those papers reporting the effects of a manipulation on thyroid hormone levels, only baseline levels were considered. A conversion factor was used to record all concentrations in International System (SI) units of nmol/L. The mean T_3 or mean T_4 concentration was sometimes recorded in the tables as the mean of more than 1

reported mean, regardless of the number of horses per group. The range was recorded in the tables as the minimum and maximum levels reported in a paper, or as ± 1.96 standard deviations of the mean, or as the lowest and highest mean values reported from many groups of horses. The standard deviation was sometimes calculated from the reported standard error of the mean.

2.1.3 Factors affecting triiodothyronine and thyroxine concentrations

2.1.3.1 Inherent variability

The range of T_3 and T_4 concentrations in the blood of healthy adult horses is very wide (Appendices A and B) and can include concentrations below the level of detection of the assay as was experienced in studies by Morris and Garcia (1983) and Blackmore et al. (1978). Researchers who establish a normal range or 95% confidence interval based on small numbers of samples from a small number of individuals run the risk of defining the interval too narrowly. As a result, the possibility exists of having a normal value interpreted as abnormal and, therefore, diagnosing a horse as hypothyroid when it is euthyroid.

2.1.3.2 Age

There is agreement that neonatal foals have markedly elevated thyroid hormone levels when compared to adults. During the first 48 hours of life, both T_3 and T_4 levels in foals are typically between 6 and 15 nmol/L and 200 and 600 nmol/L, respectively

(Appendices C and D). After about 1 week of age, T_3 and T_4 levels begin to decline, but remain elevated in comparison to adults until about 1 year of age.

Few have investigated the variation in thyroid hormone levels with age in mature horses. Kallfelz and Erali (1973) found no significant difference in T_4 between 1 year old horses and those 6 to 11 years of age. Thomas and Adams (1978) reported no significant difference in T_4 levels in horses 1 year of age and older, and Chen and Riley (1981) found no significant difference in either T_3 or T_4 levels in horses between 2 and 25 years of age. However, Kallfelz and Erali (1973) as well as Chen and Riley (1981) felt there was a trend toward decreasing thyroid hormone concentrations with increasing age.

2.1.3.3 Breed

Remarkably, little work has been published which examined the potential variation of thyroid hormone levels as a result of breed. No such relationship was found by either Thomas and Adams (1978) or Chen and Riley (1981), but limited numbers of animals were involved in each investigation.

2.1.3.4 Sex

It has been generally assumed that there is no difference in thyroid hormone concentrations due to sex. Thomas and Adams (1978) as well as Chen and Riley (1981) concluded that there was no significant differences in T_4 due to sex. However, Chen and Riley (1981) found a tendency for stallions to have higher T_4 levels and statistically

increased levels of T_3 when compared to either mares or geldings. Reap et al. (1978), although working with small numbers, showed stallions had statistically higher levels of both T_3 and T_4 compared to mares.

2.1.3.5 Reproductive status

Kelly et al. (1974) and Johnson (1986) reported that T_4 levels did not change significantly in relation to the stage of the estrous cycle in mares, and Johnson (1986) found the same to be true for T_3. Kelly et al. (1974), however, noted a tendency for T_4 levels to decrease after mares ovulated. Katovich et al. (1974) found that mares in early and late pregnancy had T_4 levels similar to non-pregnant mares, while Flisińska-Bojanowska et al. (1991) observed that T_3 levels in pregnant mares declined steadily throughout gestation and were significantly lower in the second half of pregnancy compared to the first half. Katovich et al. (1974) also documented that lactating mares had significantly lower T_4 concentrations than non-pregnant and non-lactating mares. Ferlazzo et al. (1988) found no difference in T_4 levels in mares followed from 15 days before foaling until 30 days after foaling. However, a small but statistically significant increase in T_3 was present 30 days after foaling compared to 15 days before foaling, at foaling, and 15 days after foaling.

2.1.3.6 Daily rhythm

The possible presence of a diurnal or circadian rhythm of thyroid hormone concentrations in horses has been studied with conflicting results. Morris and Garcia

(1983) were the first to report that adult horses showed a mild diurnal variation in T_4 levels, with the highest levels present between 17:00 hours and 20:00 hours and the lowest levels present at 8:00 hours. They found no diurnal variation in T_3 levels. Duckett et al. (1989) observed that T_4 concentrations in 10 geldings peaked around 16:00 hours and were significantly higher than the lowest levels experienced around 4:00 hours. In their study, T_3 levels also varied with time, reaching a peak around 8:00 hours which was statistically higher than the lowest concentrations present around 24:00 hours. Conflicting with these results were those of Flisińska-Bojanowska et al. (1991) who, working with barren and pregnant mares, found no daily rhythm in T_4 levels, but a diurnal variation in T_3 levels with peak levels around 14:00 hours. Finally, the horses studied by Hood et al. (1987) showed no significant diurnal variation in T_3 or T_4, and Sojka et al. (1993) did not find significant differences in the T_3 or T_4 levels between samples collected around 9:00 hours and 18:00 hours.

2.1.3.7 Seasonal rhythm

There is general agreement among those who have studied it, that circulating thyroid hormone levels vary with the season. However, there is a lack of consensus about the nature of the seasonal change and the stimulus for these fluctuations. For example, McBride et al. (1985) noted that both T_3 and T_4 levels increased during a 2 month period of winter but this change was not attributed to changes in ambient temperature. Johnson (1986) found a seasonal variation in T_4 levels of barren mares in which levels were greatest in October and November. While there was no apparent

relationship between T_4 levels and temperature, there was a slight but significant inverse relationship with photoperiod. In contrast, Johnson (1986) also reported that T_3 levels had a highly significant inverse relationship with temperature. Flisińska-Bojanowska et al. (1991) reported seasonal cyclicity of T_3 levels in both barren and pregnant mares with the highest levels present in the middle of the summer (near the end of July). Interestingly, while there was also a seasonal cyclicity of T_4 levels, peak levels in pregnant mares occurred in December and in barren mares it occurred in March. Finally, Katovich et al. (1974) found no difference in T_4 levels in horses between late winter and summer.

2.1.3.8 Abrupt temperature change

The limited information available about the response of the horse to abrupt changes in ambient temperature are contradictory. Katovich et al. (1974) observed that mares exposed to a sudden drop of ambient temperature, to near freezing for 2 days in November, had elevated T_4 levels compared to their levels in July. McBride et al. (1985) saw no change in either T_3 or T_4 levels in horses placed in artificially created cold temperatures for 6 hours.

2.1.3.9 Diet

Feeding patterns and the composition of the diet has been shown to influence thyroid hormone levels in horses. Recently, Messer et al. (1995b) demonstrated that both T_3 and T_4 levels were substantially reduced 1 and 2 days, respectively, after food

deprivation and were lowest after 2 and 4 days, respectively, of fasting. Within 3 days of returning to normal food consumption, T_4 levels in these horses returned to pre-fast levels while T_3 levels changed very little.

In 1984, Glade et al. found that T_4 levels in 6 to 8 month old horses increased following ingestion of a meal containing either 80% or 160% of National Research Council recommendations for energy and protein. However, they observed that the magnitude and duration of this increase was greater in the horses receiving the meal lower in energy and protein. In a subsequent study, again using 6 to 8 month old horses, Glade and Reimers (1985) confirmed that T_4 levels increase for several hours after the consumption of meals containing low (70% of National Research Council recommendations) and moderate (100% of National Research Council recommendations) amounts of energy in the form of carbohydrates but that T_4 levels decreased with the consumption of meals high (130% or National Research Council recommendations) in energy. Conversely, T_3 levels were unaffected by the ingestion of low energy diets but increased after these horses ate moderate or high energy meals. However, after being fed the same diets for 6 months the postprandial effect of meal consumption on thyroid hormone levels was no longer present. In 1987, Glade and Luba demonstrated a rapid rise in both T_3 and T_4 after the nasogastric infusion of carbohydrates, specifically 2.6 or 3.4 g/kg sucrose, in 6 month old horses. However, the nasogastric infusion of protein, specifically 1.3 or 1.7 g/kg casein, did not affect T_3 or T_4 levels. Contrary to these findings, Hood et al. (1987) produced a significant drop in both T_3 and T_4 values after nasogastric administration of food sufficiently high in

carbohydrates to produce acute laminitis. Sticker et al. (1995) fed mature horses diets restricted in protein, or energy, or both for about a month and found no alteration in either T_3 and T_4 concentrations in blood samples drawn daily.

Silva et al. (1987) found that T_4 levels were lower and T_3 levels higher when mares were fed a ration which was excessive in iodine compared to levels 7 weeks after the excessive iodine was removed from the diet. The role of endophyte infested fescue grass in the reduction of T_4 but not T_3 levels in mares (Thompson et al. 1986) is discussed below in relation to the effects of concurrent disease on thyroid hormone levels.

2.1.3.10 Exercise

Garcia and Beech (1986) examined the immediate effects of exercise on thyroid hormone levels. They demonstrated that the T_3 and T_4 concentrations in horses were unchanged immediately after swimming, but were slightly and significantly elevated 1 hour later. The general effects of exercise and fitness on thyroid hormones was examined by Takagi et al. (1974). They found that T_4 levels in young adult horses were decreased in the middle of a training program and increased late in a training program compared to levels measured early in the training program.

2.1.3.11 Medication

Morris and Garcia (1983) showed that T_3 and T_4 concentrations were significantly reduced in horses which received phenylbutazone for 5 days. Sojka et al.

(1993) found that phenylbutazone administered for 7 days lowered T_3 and T_4 levels but the changes in T_3 values were not significant. In contrast, short term administration of 2 different anabolic steroids did not change T_3 or T_4 concentrations (Morris and Garcia 1985) and neither did 5 consecutive days of dexamethasone treatment (Messer et al. 1995a). Not surprisingly, Chen et al. (1984) produced increases in T_3 for about 2 hours and increases in T_4 for about 24 hours following oral administration of about 20 μg/kg of L-thyroxine.

2.1.3.12 Concurrent disease

A reduction in circulating thyroid hormone levels in the presence of severe non-thyroidal illness is recognized in people (Utiger 1980; Wartofsky and Burman 1982; Larsen and Ingbar 1992) and in dogs (Ferguson 1988; Peterson and Ferguson 1989; Chastain and Panciera 1995) and is referred to as the euthyroid sick syndrome. Lists of known causes of the euthyroid sick syndrome in people and dogs are presented in Table 2.1. While definitive evidence is lacking, the reduction of circulating thyroid hormones is believed to be a beneficial adaptation of the body to limit the loss of protein and dampen the metabolic rate during illness and other catabolic states (Utiger 1980; Wartofsky and Burman 1982). To the best of the knowledge of this author, there has been no work to confirm the presence of the euthyroid sick syndrome in horses; however others (Sojka 1993) have taken the position that there is no reason not to suspect that the syndrome exists, and suggest horses with pituitary adenomas exhibit the euthyroid sick syndrome (Sojka 1996).

Table 2.1. Known causes of the euthyroid sick syndrome in people and dogs

People[a]	Dogs[b]
Starvation, fasting or malnutrition	Fasting, calorie or protein deficiency
Surgery (or postoperative state)	Surgery (or anesthesia)
Acute and chronic illness, such as	Debilitating disease, such as
trauma	pyoderma
thermal injury	diabetes mellitus
myocardial infarction	renal failure
infections	hepatic disease
diabetes mellitus	hyperadrenocorticism
renal dysfunction or disease	hypoadrenocorticism
some types of hepatic dysfunction or disease	intervertebral disc disease
pulmonary disease	some neuromuscular diseases
chronic arthritis	
malignancy	

[a]from Utiger 1980; Wartofsky and Burman 1982; Larsen and Ingbar 1992
[b]from Peterson and Ferguson 1989; Chastain and Panciera 1995

In 1979, Waldron-Mease identified a group of racing horses which had poor appetites, weakness, a reluctance to work, stiff gaits and substandard racing performance manifested as inconsistency or decreased endurance. These horses had low T_4 levels but all responded well to TSH administration. Beech and Garcia (1985) found that horses with pituitary adenomas had lower T_4 levels than control horses, but that T_3 levels were not affected. Hood et al. (1987) demonstrated that horses with chronic laminitis had elevated T_3 levels compared to healthy controls, while no difference was seen in T_4 concentrations. Furr et al. (1992) found a tendency for foals less than 30 days of age, hospitalized and stressed by a variety of diseases, to be more likely to develop gastric ulcers and to have lower T_3 and T_4 levels compared to age matched controls.

Thompson et al. (1986) found that agalactic mares had lower T_4 concentrations than normally lactating mares, while the 2 groups had similar levels of T_3. All the mares in that study had grazed fescue grass for a variable period of time during gestation. While the authors mention the possibility that endophyte infestations of the fescue grass may have had a role in producing the agalactia (Putnam et al. 1991; Boosinger et al. 1995), the rate of exposure of the mares was not determined.

2.1.3.13 Summary

These discussions have tried to summarize the many attempts to define the parameters of thyroid hormone levels in horses. While some of the studies reviewed were principally descriptive, others were interested in identifying events or factors which might alter thyroid hormone levels. Based on this review, this author concluded

that resting thyroid hormone levels are influenced by a number of factors, some of which might be present concurrently, and that the interpretation of lower than expected levels of T_3 and T_4, alone or together, should not be considered diagnostic of hypothyroidism in the horse. In fact, it should remembered that thyroid hormone concentrations in normal horses were occasionally lower than the limit of detection of the assay. For these reasons, thyroid function tests, rather than resting T_3 or T_4 levels, are currently the best means of diagnosing thyroid dysfunction in the horse.

2.1.4 Factors which may influence the interpretation of the thyroid-stimulating hormone response test and the thyrotropin-releasing hormone response test

The 2 function tests presently promoted for use in diagnosing hypothyroidism are based on the response of a horse to either TSH or TRH administration (Beech 1987; Harris et al. 1992; Messer 1993, 1994; Sojka and Levy 1995). However, as pointed out by Dybdal (1996), the variety of published protocols have consistently summarized the response of normal horses to TSH or TRH administration and then assumed that any horse that fails to respond in a similar manner is hypothyroid. Rarely has similar testing been performed on horses known to be hypothyroid to support the arbitrarily determined criteria of euthyroidism or hypothyroidism.

This argument notwithstanding, these tests presumably have the advantage of overcoming problems of interpretation associated with the inherent variability of resting T_3 and T_4 levels outlined above, the effects of concurrent nonthyroidal disease

(Waldron-Mease 1979; Beech and Garcia 1985), and depressed hormone levels created by phenylbutazone administration (Morris and Garcia 1983). It is important to recognize that the response of a horse during a stimulation test will depend on the individual animal, whether TSH or TRH is used, and the dose and route of administration of the exogenous hormone (Morris and Garcia 1983; Held and Oliver 1984; Beech and Garcia 1985; Chen and Li 1985; Oliver and Held 1985; Lothrop and Nolan 1986; Harris et al. 1992).

Another factor which may affect the magnitude of the response a horse exhibits following TSH or TRH administration and which, therefore, may influence the interpretation of a response test, is the actual biologic activity of the preparation administered. While issues of this nature have received some attention in the field of reproductive endocrinology (Newcomb et al. 1979; Murphy et al. 1984), questions about the consistency in biological activity of TSH and TRH preparations between different manufacturers and between different batches from the same manufacture do not appear to have received any attention.

Personal communication with members of the Bureau of Human Prescription Drugs, Health Protection Branch, Health and Welfare Canada, indicated that hormones, such as the bovine TSH[a] used in these studies, which are licensed for use in human clinical endocrinology, may vary ± 20% in biological activity. The quality control personnel of the supplier of bovine TSH[a] used in these studies stated (personal communication) that their product varied by ± 10% in biological activity. Lastly, the technical services staff of another supplier of bovine TSH[b] suggested (personal

communication) that their product varied approximately ± 5% in biological activity and that individual vials of bovine TSH[b] rarely contain less hormone, but may contain over 30% more hormone. Based on these figures, different horses intended to receive the same dose of TSH may receive doses that differ in biological activity by 20 to 30%.

2.2 Validation of the Fluorescence Polarization Immunoassay to Measure Thyroid Hormones of Horses

2.2.1 Introduction

The validation of a hormone assay refers to the process of establishing how well the assay measures the hormone it is designed to measure. The concept of validity is also referred to as accuracy or specificity by some. Three criteria need to be met in validating a nontraditional bioassay and include: (1) that hormone added to the biological fluid to be assayed be measured quantitatively; (2) that the apparent hormone content of an unknown sample must decrease linearly with dilution, or alternatively, a dilution curve of the unknown sample must be superposable on a dilution curve of a standard solution over a wide range of concentrations; and (3) that the hormone not be detected in a sample of a relevant biological fluid that does not contain any hormone (Reimers et al. 1981; Chard 1987; Smith 1991; Yalow 1992).

Another aspect regarding the quality of a hormone assay is that of precision or reproducibility. Precision in this context is a measure of the variation observed between repeated determinations of the same sample. The amount of variation that exists between one performance of the assay to another, i.e., from day to day or between

different "runs", is referred to as the between-assay or inter-assay variation. The amount of variation present during continuously performed assays, i.e., during the same "run", is referred to as the within-assay or intra-assay variation. A measure of these sources of variation is the coefficient of variation (CV) which is calculated as the standard deviation divided by the mean of the repeated measurements of the same sample, and multiplied by 100 (Reimers et al. 1981; Chard 1991).

Two other concerns about the usefulness of an assay are termed sensitivity and cross-reactivity. The sensitivity of an assay, sometimes referred to as absolute sensitivity or detectability, is defined as the minimal detection limit of an assay, or the least concentration of hormone which can be distinguished from a sample containing no hormone. The degree to which the measurement of one hormone is interfered with by the presence of a second hormone or chemical is referred to as cross-reactivity or specificity (Reimers et al. 1981; Chard 1991; Yalow 1992). Issues of sensitivity and cross-reactivity of FPIA will addressed in the discussion.

2.2.2 Materials and methods

The FPIA under investigation was commercially available and the manufacturer supplied standard solutions containing 0.0, 19.3, 38.6, 77.2, 154.5, and 231.7 nmol/L of T_4. Serum from 1 adult horse and 2 equine fetuses served as samples with unknown T_4 concentrations. The adult horse (M15) was 3 to 6 years old, healthy, female, and had not received any medication for 30 days prior to sample collection. Both fetuses were sampled at a commercial abattoir within 30 minutes of slaughter, and both appeared to

be normally developed. Using criteria assembled by Roberts (1971; 1986), 1 fetus (F-20-2) was estimated to be 5 months gestation, and the other (F-20-6) was estimated to be 11 months gestation.

The standard T_4 curve was constructed by plotting the polarization values for duplicate assays of each of the standard solutions. The T_4 curve of horse serum and added hormone was constructed by plotting the polarization values for duplicate assays of the adult horse serum (M15), and the adult horse serum to which appropriate amounts of standard solution was added to increase the T_4 content by 15.5, 30.9, 61.8, 70.2, and 154.0 nmol/L. Two T_4 curves of various dilutions of fetal horse serum were constructed by plotting the polarization values for duplicate assays of each of the 2 fetal serum samples (F-20-2 and F-20-6), as well as 1:1, 1:3, and 1:7 dilutions of each sample using the standard sample containing 0.0 nmol/L as the diluent. Statistical and graphing software and a personal computer were used to calculate the strength and direction of the linear relationship, i.e., correlation coefficient (r), between T_4 concentrations and polarization, and the best fitting line for the data was calculated and described in the standard formula of $y = A + Bx$ (where A = the y-intercept and B = the slope).

The inter-assay CV for the T_4 assay was calculated on 15 replicate samples of 4 different solutions containing 21.1, 58.7, 101.0, and 188.0 nmol/L. The intra-assay CV for the T_4 assay was calculated on 3 replicate samples of 3 different solutions containing 21.5, 50.0, and 95.0 nmol/L. The inter-assay CV for the T_3 assay was calculated on 10 replicate samples of 4 different standard solutions containing 0.84, 2.47, 4.63, and 8.06 nmol/L. An intra-assay CV for the T_3 assay was not calculated.

2.2.3 Results

The mean T_4 concentrations of samples from M15, F-20-2, and F-20-6 were 32, 128, and 244 nmol/L, respectively. The results of the assay of the standard solutions, i.e., the standard curve, and of hormone added to serum from M15 appear in Figure 2.1. The results of the assay of serial dilutions of serum from F-20-2 and F-20-6, and the standard curve appear in Figure 2.2. The correlation coefficient (r) for the 4 analyses were all close to 1 and the equations describing each linear relation were similar, and are presented in Figures 2.1 and 2.2.

The inter-assay CV for the 4 serum samples with T_4 concentrations between 21.1 to 188.0 nmol/L ranged from 5.0 to 8.5%; the intra-assay CV ranged from 2.6 to 7.5%. The inter-assay CV for the 4 serum samples with mean T_3 concentrations between 0.84 to 8.06 nmol/L ranged from 6.7 to 11.5%.

2.2.4 Discussion

The FPIA used in these assays demonstrated the qualities of a valid and useful assay of thyroid hormones in horses. Samples of adult horse serum with added T_4 and serial dilutions of equine fetal serum showed a parallel relationship with, or were superposable upon, the standard curve.

The manufacturer of the assay has reported that the sensitivity of the T_4 assay was 5 nmol/L, that measurements of T_4 were unreliable at concentrations approaching 232 nmol/L, and that the cross-reactivity of the T_4 assay with T_3 was 8%. The manufacturer has also reported that the sensitivity of the T_3 assay was 0.5 nmol/L, that

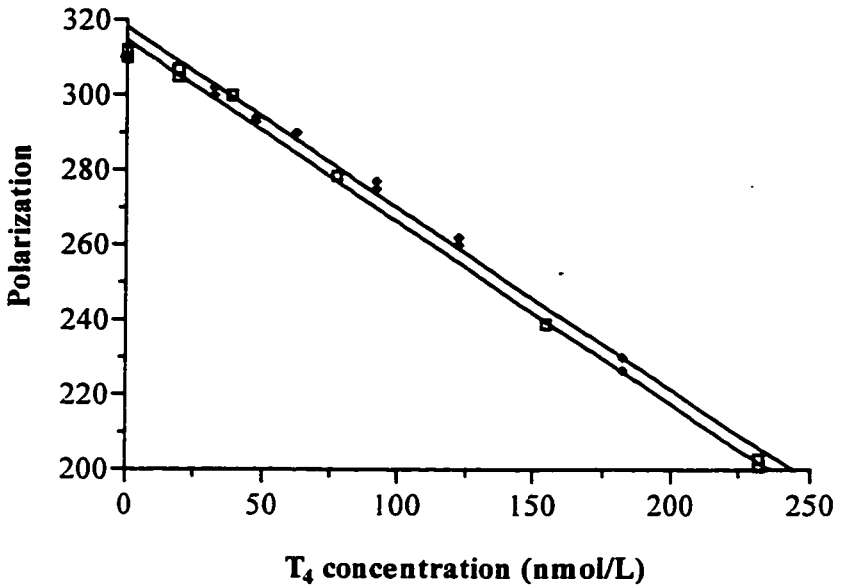

Figure 2.1. Validation of the fluorescence polarization immunoassay using horse serum with added thyroxine (T_4). The line generated from assays of standard solutions (the standard curve) is superposable upon the line generated from assays of horse serum, with an unknown concentration of T_4, and the horse serum after the addition of known amounts of T_4.

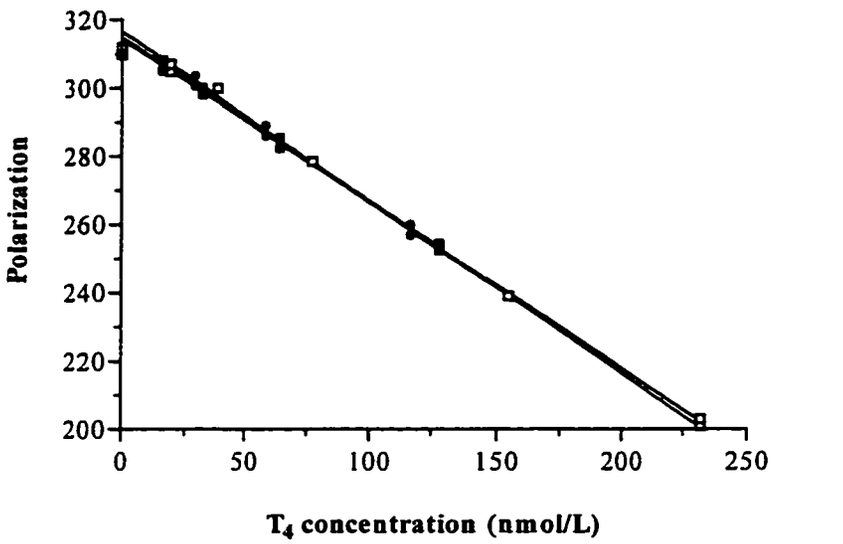

Figure 2.2. Validation of the fluorescence polarization immunoassy using diluted fetal horse serum. The line generated from assays of standard solutions (the standard curve) is superposable upon lines generated from assays of fetal horse sera, with unknown concentrations of thyroxine (T_4), and the fetal sera which had been diluted 1:1, 1:3, and 1:7 with a standard solution containing no T_4.

measurements of T_3 were unreliable at concentrations approaching 9.2 nmol/L, and that the cross-reactivity of the T_3 assay with T_4 was 0.23%.

2.3 The Effects of Delayed Serum Separation and Moderate Term Storage at Room Temperature on the Measurement of Thyroid Hormones

2.3.1 Introduction

This trial was conducted to prepare for the study of thyroid function in normal mares and foals. Because client-owned mares foaling on their home farms were to be used, it was important to determine if any aspect of sample handling affected the measurement of thyroid hormones using the FPIA. Specifically, this trial examined the effects of keeping serum on the clot and at room temperature for up to 24 hours on the measurement of T_3 and T_4 concentrations using the FPIA.

2.3.2 Materials and methods

Four sterile, evacuated, 10 ml, glass tubes with no additive were filled via venipuncture with blood from each of 16 different horses. The horses included 6 adult barren mares, 6 adult mares which had foaled within 12 hours prior to blood collection, 2 adult stallions, and 2 foals less than 12 hours old. Blood was allowed to clot at room temperature (20°C to 24°C) and at 1 hour, 6 hours, 12 hours, and 24 hours after collection, 1 tube of blood from each horse was centrifuged for 10 minutes at 3,000 revolutions per minute (1,000 × *g*). After centrifugation, serum was removed from the

clot using a plastic pipette, placed in a plastic vial with a watertight stopper and frozen at −20°C for 2 to 4 weeks in a non-frost-free freezer, until assayed. Assays for T_3 and T_4 concentrations were conducted, with the operator unaware of the treatment of each sample. To avoid inter-assay variation, all 64 samples were assayed in 2 batches with all samples from the same animal assayed as part of the same batch. The results were compared using Friedman nonparametric repeated measures tests. Any hormone concentration which was less than the limit of detection of the assay was assigned a value that was the arithmetic mid-point between zero and the lowest detectable concentration. Therefore, T_3 and T_4 concentrations below the limit of detection of the assay were assigned values of 0.25 nmol/L and 2.5 nmol/L, respectively.

2.3.3 Results

Five of 64 T_3 values were below 0.5 nmol/L and 2 low values were reported for each of 2 horses. Only 3 of 64 T_4 values were below 5 nmol/L and all were associated with samples from the same animal.

There were no apparent trends or statistically significant differences ($P = 0.7$) associated with the measured concentrations of T_3 or T_4 and the amount of time taken (up to 24 hours) to separate serum from the blood clot prior to freezing (Table 2.2).

2.3.4 Discussion

The results of this trial demonstrated that leaving equine serum in contact with the clot, at room temperature, for as long as 24 hours after collection had no effect on

Table 2.2. The median and range of triiodothyronine and thyroxine concentrations in equine serum samples left in contact with the clot at room temperature for up to 24 hours after collection.

	Time serum left in contact with clot			
Hormone	1 Hour	6 Hours	12 Hours	24 Hours
T_3 (nmol/L)[a]	1.06	1.18	1.11	1.12
Range[b]	0.52 - 12.70	0.25 - 13.30	0.51 - 12.80	0.25 - 13.70
T_4 (nmol/L)[c]	35.0	28.5	33.5	32.0
Range[b]	2.5 - 354.0	2.5 - 350.0	8.0 - 354.0	2.5 - 360.0

T_3 = triiodothyronine
T_4 = thyroxine
[a]*P*-value associated with Friedman nonparametric repeated measures test of values in the row is 0.7001
[b]minimum and maximum concentrations
[c]*P*-value associated with Friedman nonparametric repeated measures test of values in the row is 0.6915

the measurement of T_3 or T_4 using the FPIA system. This finding is similar to those of Reimers et al. (1991) who found that the concentrations of T_3 and T_4 in equine plasma were not different when samples were stored at either 4°C or 20°C for 18 hours and measured by RIA. The same group has also shown that the measurement of T_4 in canine plasma or serum (Reimers et al. 1982) and of T_3 and T_4 in bovine plasma or serum (Reimers et al. 1983) is unaffected by storage at temperatures as high as 20°C for as long as 8 days. Nye et al. (1975) found that T_4 levels were unchanged in human serum samples left at room temperature for 2 weeks and measured by RIA. They also reported that T_3 levels showed a small but significant decline when samples were stored at room temperature. This change was reduced with storage of samples at 4°C.

In retrospect, it would have been useful to assess the effects of hemolysis on the measurement of thyroid hormones with our system. Previous work has shown that the measurement of T_3 and T_4 by RIA was unaffected by hemolysis (Nye et al. 1975; Reimers et al. 1982, 1991). However, the presence of hemoglobin interfered with the measurement of T_4 in human serum using the FPIA system utilized in our investigations (Symons and Vining 1985).

The most important aspect of this trial was the confirmation that, when measured with an immunoassay, T_3 and T_4 are resistant to degradation, immunologically stable, and insensitive to potential problems associated with routine specimen handling.

2.4 Thyroid-Stimulating Hormone Response Tests in Postpartum Mares and Neonatal Foals

2.4.1 Introduction

The evaluation of thyroid gland function in horses has been a concern of researchers and practitioners since the 1960s (Wilson et al. 1961; Kaneko, 1964; Irvine, 1966). However, only modest progress (Beech, 1987; Sojka & Levy, 1995) has been made in developing an accessible, reliable and reasonably priced means of assessment since this notion was expressed about 30 years ago (Irvine, 1966).

In contrast to the growing body of work concerned with thyroid function testing in adult horses, the examination of thyroid gland function in neonatal foals has received little attention (Shaftoe et al. 1988). The objectives of these studies were (1) to determine the change in T_3 and T_4 concentrations with time in response to TSH administration in postpartum mares and neonatal foals using resources available at the WCVM, and (2) to compare the results with those previously published.

2.4.2 Materials and methods

Seven horse breeders in close proximity to the WCVM agreed to participate in these studies. Using a random numbers table, 2 pregnant mares were randomly selected from all pregnant mares resident on each of the breeding farms. The 14 pregnant mares selected were all multiparous and consisted of 2 Arabian horses, 4 Quarter horses, 4 Standardbred horses, and 4 Thoroughbreds, ranging from 5 to 15 years of age. The

mares foaled spontaneously and without assistance, and had not received any medications prior to parturition.

The horse breeders notified the author when a mare had foaled. Each mare and foal were observed, received a physical examination, and had blood samples taken for a complete blood count and serum chemistry analysis. A TSH response test was performed on each mare and foal using published guidelines (Held and Oliver 1984; Shaftoe et al. 1988). Specifically, blood samples were collected from the jugular veins of the mares and the jugular veins, or occasionally, branches of the cephalic and saphenous veins, of the foals. A separate 10 IU vial of bovine TSH[a] was used for each mare and foal pair and reconstituted just prior to use. Each animal was given 5 IU of TSH[a] intravenously and blood samples collected every hour for 5 hours from foals and for 8 hours from mares. Blood samples were collected into evacuated, 10 ml, glass tubes with no additive, allowed to clot at ambient temperatures, and centrifuged for 10 minutes at 3,000 revolutions per minute (1,000 × *g*). Serum was removed from the clot using a plastic pipette, placed in a plastic vial with a watertight stopper, kept on ice for several hours before being frozen at -20°C in a non-frost-free freezer for not longer than 7 days, until assayed. Assays for T_3 and T_4 concentrations were performed at the Endocrinology Laboratory, WCVM, using the commercially available FPIA.

For the purposes of statistical analysis, T_3 and T_4 levels below the level of detection were assigned a value of 0.25 and 2.5 nmol/L, respectively. The mean and standard error of the mean of T_3 and T_4 concentrations at each time interval were calculated for mares and foals separately and variation among the means compared

using a repeated measures analysis of variance. For those analyses found to be significant ($P < 0.0001$), the mean concentrations of T_3 and T_4 after TSH administration were compared to the initial (baseline) mean concentrations using Dunnett multiple comparisons tests.

2.4.3 Results

All mares foaled between April 19 and June 3 and all TSH response tests were initiated between 6 and 18 hours after parturition. Testing of 10 mare and foal pairs was initiated during daylight hours (7:00 to 18:00 hours) and testing of 4 mare and foal pairs was initiated during the night time hours (20:00 to 23:00 hours). The data from 1 Standardbred foal was excluded from the analyses as this foal showed signs of prematurity. The results of the TSH response tests in mares are presented in Figure 2.3 and in foals presented in Figure 2.4.

In mares, baseline T_3 concentrations ranged from < 0.5 to 2.72 nmol/L with a mean of 1.10 ± 0.16 nmol/L, and T_4 concentrations ranged from < 5.0 to 40 nmol/L with a mean of 24.8 ± 2.8 nmol/L. All mares had increased levels of T_3 and T_4 following TSH administration. All mares in this study more than doubled their T_3 concentrations, with 12 of 14 mares tripling their T_3 levels within 3 hours after TSH administration. The mean levels of T_3 after 1, 2, 3, and 4 hours were significantly higher ($P < 0.01$) than the mean baseline level. Maximal levels of T_3 in each mare were demonstrated within 4 hours, with 6 of 14 mares showing this peak after 2 hours. The mean peak T_3 concentration was at 2 hours (3.86 ± 0.58 nmol/L).

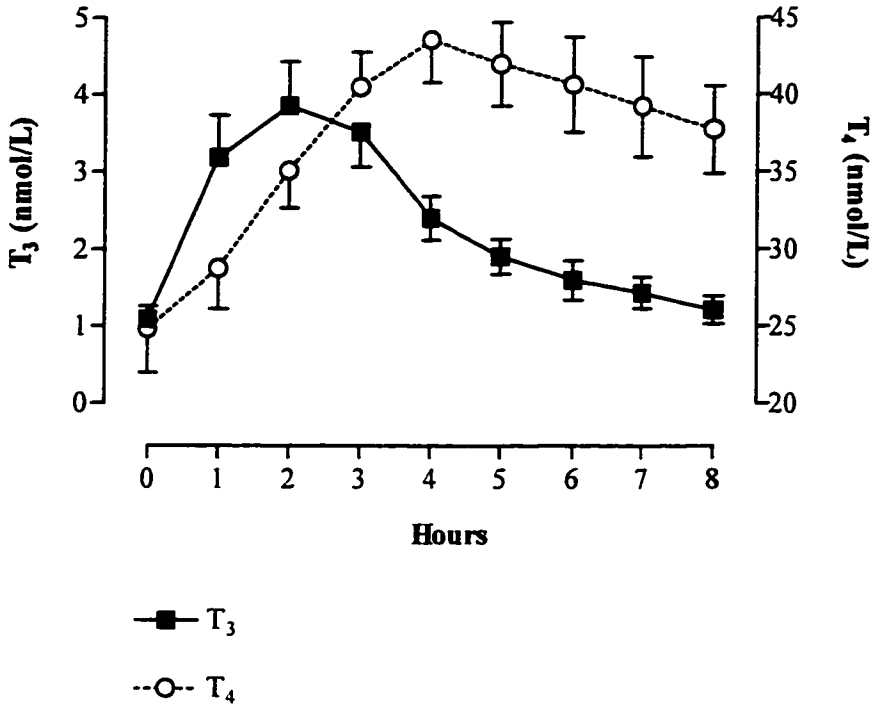

Figure 2.3. Mean (± standard error) serum triiodothyronine (T_3) and thyroxine (T_4) concentrations in postpartum mares before and for 8 hours after thyroid-stimulating hormone administration.

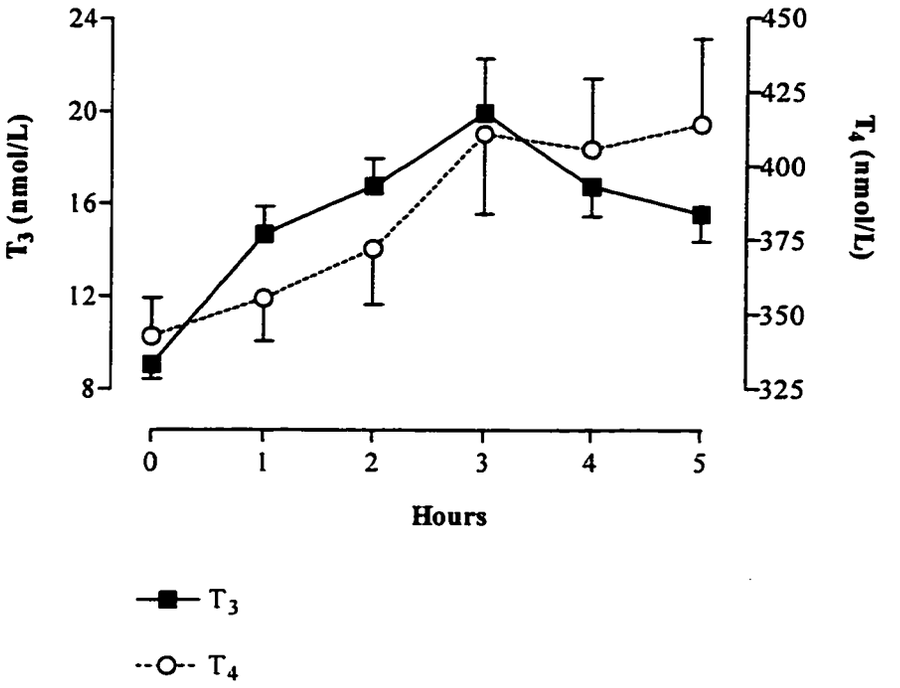

Figure 2.4. Mean (± standard error) serum triiodothyronine (T_3) and thyroxine (T_4) concentrations in newborn foals before and for 5 hours after thyroid-stimulating hormone administration.

The mean levels of T_4 at hours 2 through 8 were significantly higher ($P < 0.01$) than the mean baseline level. Maximal levels of T_4 in each mare was observed between 3 to 7 hours after TSH administration, with 7 mares showing this peak at 4 hours. The mean peak T_4 concentration at 4 hours was 43.8 ± 2.8 nmol/L.

In foals, baseline T_3 concentrations ranged from 5.86 to 12.85 nmol/L with a mean of 9.08 ± 0.64 nmol/L, and T_4 concentrations ranged from 254.0 to 435.0 nmol/L with a mean of 342.8 ± 13.0 nmol/L. All foals had increased levels of T_3 and T_4 following TSH administration. The mean levels of T_3 at each of 5 hourly points after TSH administration were significantly higher ($P < 0.01$) than the mean baseline level. Maximal levels of T_3 in each foal was between 1.2 and 7.2 times (median = 1.9 times, mean = 2.4 times) the baseline levels and was observed within 4 hours of TSH administration, with 7 of 13 foals showing this peak at 3 hours. The mean peak T_3 concentration was at 3 hours (19.9 ± 2.3 nmol/L).

The mean levels of T_4 in foals at 3, 4, and 5 hours after TSH administration were significantly higher ($P < 0.01$) than the mean baseline level with the concentrations very similar, at 410 ± 5 nmol/L. However, assessing changes in T_4 concentrations of individual foals was difficult. Maximal levels of T_4 in each foal was attained at each of the 5 hour time intervals after TSH administration examined, with the maximum increase ranging from 5 to 68% (median = 16%, mean = 25%).

Examination of Appendices A, B, C, and D indicates that concentrations of T_3 and T_4 for both the mares and foals used in the current study are similar to those reported previously. The finding that the mean baseline T_3 levels of foals was 8.25

times higher than that of mares and the mean baseline T_4 levels of foals was 13.8 times higher is also consistent with published information.

2.4.4 Discussion

Similar to the results reported by Messer et al. (1995a), the mares in this study could be placed in 1 of 2 groups relative to their response to TSH administration. Six mares in this study had baseline T_4 concentrations ranging from < 5 to 26 nmol/L and demonstrated a 2 to 5 fold elevation of their T_4 levels. The other 8 mares, with higher baseline levels of 25 to 40 nmol/L, failed to produce peak T_4 concentrations that were double their baseline, demonstrating an increase in T_4 levels between 1.3 and 1.9 fold. A review of the data reported by Held and Oliver (1984) reveals a similar finding. Specifically, 8 of the 10 horses undergoing a similar TSH response test were found to at least double their baseline T_4 levels. The remaining 2 horses did not achieve the same proportion of increase, but they had 2 of the 3 highest baseline values of 27 and 37 ng/ml or about 35 and 48 nmol/L, respectively.

In this study, the magnitude of the elevation in T_4 concentrations in response to TSH administration did not appear to be related to the breed of the mare, the time elapsed after foaling, or the time of day the test began.

As with the horses studied by Messer et al. (1995a), the mares in this study which failed to double their T_4 concentrations following TSH administration could not be considered hypothyroid. These mares had no clinical evidence of hypothyroidism, demonstrated the highest baseline T_4 levels and, following TSH administration,

demonstrated a 30 to 90% increase in their T_4 levels and showed an elevation in T_3 levels of 235 to over 1,000%. These findings, together with those of Messer et al. (1995a) and Oliver and Held (Held and Oliver 1984; Oliver and Held 1985), suggest that it is appropriate to reevaluate the criteria used to diagnose hypothyroidism in the horse before abandoning the TSH response test as a diagnostic tool.

It has been suggested (Messer et al. 1995a), that since some horses, which are clearly euthyroid, fail to produce a doubling of their baseline levels of T_4 following TSH administration, that the TSH response test may not be as valuable a tool as once thought for diagnosing hypothyroidism in horses. Contrary to this view, this author believes the requirement for a horse to double its baseline concentration of T_4 before it is considered euthyroid is an arbitrary one which has been selected primarily because of the ease in calculating a two-fold increase.

It would seem to be more proficient to only use TSH or TRH response tests on horses already suspected of being hypothyroid based on appropriate clinical signs, and to assess the magnitude of the response to TSH or TRH administration in reference to the dose and route of the exogenous hormone administered, as well as the baseline concentrations of T_3 and T_4 relative to the normal range.

The data concerning the response of newborn foals to TSH administration generally agrees with the limited data already published (Shaftoe et al. 1988). The baseline and peak concentrations of both T_3 and T_4 were generally higher in the foals in this study compared to those of Shaftoe et al. (1988), however the magnitude of the response of the foals in this study was greater. The pattern of the response and the fact

the response was statistically significant is common to both studies and supports the use of the TSH response test as a means of assessing thyroid function in newborn foals. However, because of the limited increase in T_4 concentrations seen in foals and the variability in the time of the peak response, it is recommended to monitor both T_3 and T_4 levels during TSH response tests in foals.

3.0 CHARACTERIZATION OF THE CONGENITAL HYPOTHYROIDISM AND DYSMATURITY SYNDROME OF FOALS[1]

3.1 Introduction

The lesions typifying and defining a foal as affected with the syndrome of thyroid gland hyperplasia and musculoskeletal deformities (TH-MSD) have been described in Chapter 1 and elsewhere (McLaughlin and Doige 1981; McLaughlin et al. 1986; Allen et al. 1994). Because the musculoskeletal lesions of the TH-MSD syndrome occurred concurrently with congenital thyroid gland hyperplasia it was suspected that the musculoskeletal lesions resulted from abnormal perinatal thyroid function. While some evidence exists to suggest that foals with the TH-MSD syndrome were hypothyroid (McLaughlin and Doige 1982; McLaughlin et al. 1986), currently accepted diagnostic tests of thyroid function in foals (see Chapter 2.0) have not been performed on affected foals. The purpose of the investigations described herein were (1) to assess thyroid function in foals with musculoskeletal lesions consistent with the TH-MSD syndrome, and (2) to review the histories, clinical findings, and postmortem lesions present in affected foals. In describing the syndrome more fully, it was anticipated that understanding might be gained into the pathogenesis of the anomalies present and that this attempt would aid in future recognition of affected foals.

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[1] Portions of this chapter have been published (Allen et al. 1993).

3.2 Materials and Methods

3.2.1 Case selection

Twenty-five foals with lesions consistent with the TH-MSD syndrome were examined by veterinarians of the Field Service, Large Animal Clinic, or Diagnostic Laboratory of the Western College of Veterinary Medicine (WCVM) during 1990 through 1995, inclusive. Each foal was also examined by the investigator ante mortem, postmortem, or both. Nine of the first 13 foals examined were subjected to a thyroid-stimulating hormone (TSH) response test. All of the foals were less than 48 hours of age and 6 foals had not received any drug therapy prior to the initiation of the TSH response test.

3.2.2 Thyroid-stimulating hormone response tests

Whole blood samples were collected in glass tubes prior to the intravenous injection of 5 International Units (IU) of bovine TSH[a], as well as 3 and 4 hours after the injection of TSH[a]. Blood was allowed to clot at room temperature, the serum separated by centrifugation, and placed in plastic vials with water tight caps at -20°C until assayed. Triiodothyronine (T_3) and thyroxine (T_4) levels were determined by fluorescence polarization immunoassay (FPIA).

3.2.3 Necropsy and histology

Necropsies were performed on 22 of the foals following death or euthanasia. Thyroid glands were fixed in 10% neutral buffered formalin, embedded in paraffin,

sectioned at 4 to 6 microns, and stained with hematoxylin and eosin and with the periodic acid-Schiff reaction.

3.2.4 Radiography

Radiographs of the carpi and tarsi were made as part of the clinical or postmortem examination of all foals. The degree of ossification was assessed with the aid of a skeletal ossification index, modified from previously published material (Adams 1990; Adams and Poulos 1987, 1988), which is presented in Table 3.1.

3.2.5 Analyses

Descriptive statistics including the mean and mode were calculated for characteristics of foals that could be expressed as a continuous variable. The range was expressed as the minimum and maximum values reported. Clinical or postmortem findings of a categorical nature were summarized and expressed as a frequency.

The results of baseline serum thyroid hormone assays and of TSH response tests were compared to those obtained from normal, fullterm foals from the WCVM practice area reported in Chapter 2.0. Group means were compared at specific points in time (0, 3, and 4 hours) using an unpaired *t*-test.

3.3 Results

The 25 foals represented 9 different breeds including light (Appaloosa, Arabian, Hanoverian, Morgan, Paint, Quarter Horse, Standardbred, Thoroughbred) and draft

Table 3.1. Criteria for grading the ossification of the carpal and tarsal regions of neonatal foals

Grade	Criteria
0	- no ossification in any of the carpal and tarsal bones of interest[a] - open proximal physes of the third metacarpi and third metatarsi (the proximal epiphyses of the third metacarpi and third metatarsi usually lacked ossification centers)
1	- ossification of at least one, but not all, of the carpal or tarsal bones of interest[a] - open proximal physes of the third metacarpi and third metatarsi
2	- some ossification of all the carpal and tarsal bones of interest[a] - open proximal physes of the third metacarpi, third metatarsi, or both
3	- small ossification centers in the carpal and tarsal bones of interest[a] producing the radiographic appearance of rounded edges and widened joint spaces - closed proximal physes of the third metacarpi and third metatarsi
4	- carpal and tarsal bones of interest[a] had a pattern of ossification resembling an adult and the radiographic appearance joint spaces was of expected width - closed proximal physes of the third metacarpi and third metatarsi

[a]radial, intermediate, ulnar, second, third and fourth carpal bones and central and third tarsal bones.

breed (Clydesdale) horses. Thirteen foals were male and 12 were female. The months of birth of the foals were distributed normally over March to July, with the peak (11 of 25) in May. None of the foals was delivered prematurely; the mean gestation was equal to 355.4 days and the range was 338 to 372 days (n = 21). Dystocia or assisted delivery of the foal was reported 9 times.

One foal was stillborn; 3 foals died of sepsis within 1 week of age in spite of therapy; 19 foals were euthanatised; 1 foal was lost to follow-up after 4 months of age; and 1 male foal reached maturity, has been used for breeding, and has produced normal offspring. Of the 19 foals euthanatised, 13 were euthanatised within 48 hours of birth, and shortly after first being examined, because of marked immaturity, sepsis, or both. Six foals survived, with treatment, for between 1 week and 3 months but were euthanatised because they failed to thrive and experienced complications associated with bandages, splints or casts applied to the legs. The frequency of other clinical signs and postmortem lesions are presented in Table 3.2.

All 25 foals had incomplete ossification of the carpal and tarsal bones which, given the normal length of gestation of these foals, was evidence of immaturity. Grades of ossification ranged from 0 to 3, with 14 foals assigned a grade of 2. In 24 of 25 foals examined, the mandible extended from 3 to 20 mm ($\bar{x}$ = 8.6 mm) rostrally beyond the maxilla (i.e., mandibular prognathism).

Flexural deformities ("contracture") of the limbs was observed in 22 of 25 affected foals. This lesion was limited to the front legs in 16 foals, and involved all 4 legs of the other 6 foals. Twenty-two foals also had hemorrhage and partial tearing, or

Table 3.2. Frequency of a clinical sign or postmortem lesion in foals diagnosed at the Western College of Veterinary Medicine between 1990 and 1995, inclusive with a syndrome of thyroid gland hyperplasia and musculoskeletal deformities.

Clinical sign or postmortem lesion	Number of foals
Incomplete ossification of cuboidal bones	25
Mandibular prognathism	24
Flexural deformities	22
Hemorrhage, or tearing, or rupture of CDET	22
Thyroid gland hyperplasia with scant colloid	21
Hypogammaglobulinemia or sepsis	16
Weak, recumbent, unable to stand	16
Poor muscle development	12
Umbilical or inguinal hernia	11
Osteopetrosis	9
Immature appearance; "silky" coat, pliable ears	8
Angular limb deformities	5
Laxity of hind leg flexor tendons	4
Rupture of other tendons of the limbs	4
Small size	4

CDET = common digital extensor tendon(s)

complete rupture, of 1 or both common digital extensor tendons. Four of these foals had similar lesions of the long digital extensor tendons of the front legs, the lateral digital extensor tendons of the hind legs, or both.

An angular limb deformity, defined as a medial or lateral deviation of the limb from the sagittal axis (also referred to as a varus or valgus deformity, respectively), was present in 5 foals. The author believes that the limb deformities were caused by joint instability brought about by laxity of the periarticular supporting tissues and the malleable quality of the incompletely ossified bones (Fretz 1980). Angular limb deformities of this nature can only be diagnosed in foals capable of standing and forced to bear their weight. The same logic is applicable in the diagnosis of laxity of the flexor tendons of the hind legs. In retrospect, the author believes that several more of the foals examined had abnormally lax joints and flexor tendons than were formally reported here.

Lax joints and tendons are clinical signs consistent with immaturity of foals. As was discussed previously, all of the TH-MSD foals had incompletely ossified or immature cuboidal bones, and 8 foals were described as having an immature appearance based on a short, soft, "silky" coat and soft, pliable ears.

Osteopetrosis, which has rarely been described in horses, was diagnosed in 9 of the 22 foals necropsied. The same diagnosis was made previously in 2 aborted fetuses with histories and lesions consistent with the TH-MSD syndrome (Allen 1995).

It was not known why 16 of the TH-MSD were continuously recumbent or unable to stand. The author believes that the inability to stand was related to weakness

(which was described by the attending clinician), flexural deformities, lax tendons and joints, or a combination of these factors. The inability to stand likely contributed directly to the failure of foals to suckle, ingest colostrum, and acquire passive immunity. The failure of passive transfer of immunity is no doubt related to the high rate of sepsis in these foals. What needs to be determined is whether TH-MSD foals have the ability to absorb antibodies through the gut if they were delivered in adequate quantities. The question of maturity of enterocytes and the small intestine is raised by the immaturity present in other body systems.

The thyroid glands of the 22 foals examined postmortem appeared normal grossly and were unremarkable in size, shape or location; however, they were considered abnormal after histologic examination. Twenty-one of the glands were diffusely hyperplastic and contained little to no colloid. The remaining thyroid gland was composed of small follicles containing scant colloid and surrounded by an increased amount of connective tissue.

The results of serum T_3 and T_4 measurements before and after TSH administration are displayed in Figures 3.1 and 3.2, respectively. The results of TSH response tests performed on the normal and healthy foals described in Chapter 2.0 are also presented for comparison. There were no apparent or statistical differences in T_3 or T_4 concentrations between TH-MSD foals that had received medication and those that had not. Therefore, all 9 foals were included in the calculation of the means and statistical comparisons with normal foals.

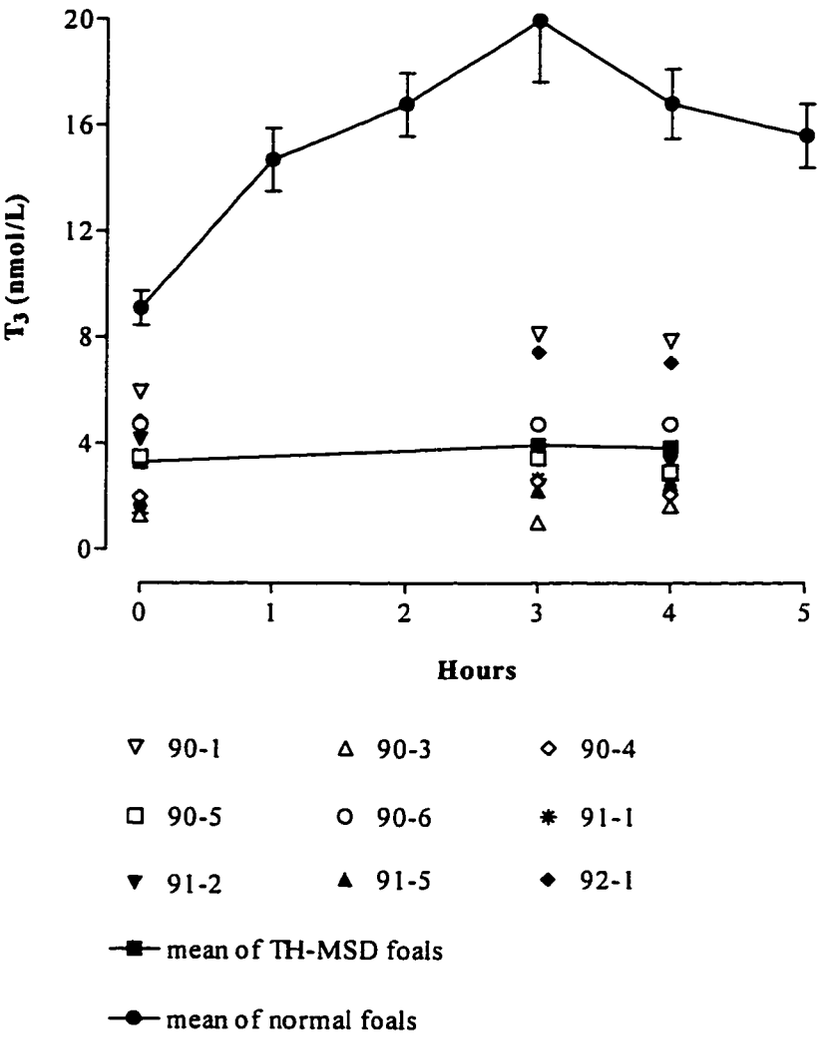

Figure 3.1. Serum triiodothyronine (T_3) concentrations in neonatal foals before and at various times after intravenous thyroid-stimulating hormone administration. The concentrations measured in 9 foals with thyroid gland hyperplasia and musculoskeletal deformities (TH-MSD) are presented individually and as mean values. The mean (± standard error) T_3 concentrations of 13 normally developed and healthy foals (see Figure 2.4) are presented for comparison. Mean values for TH-MSD and normal foals were significantly ($P < 0.0001$) different at 0, 3 and 4 hours.

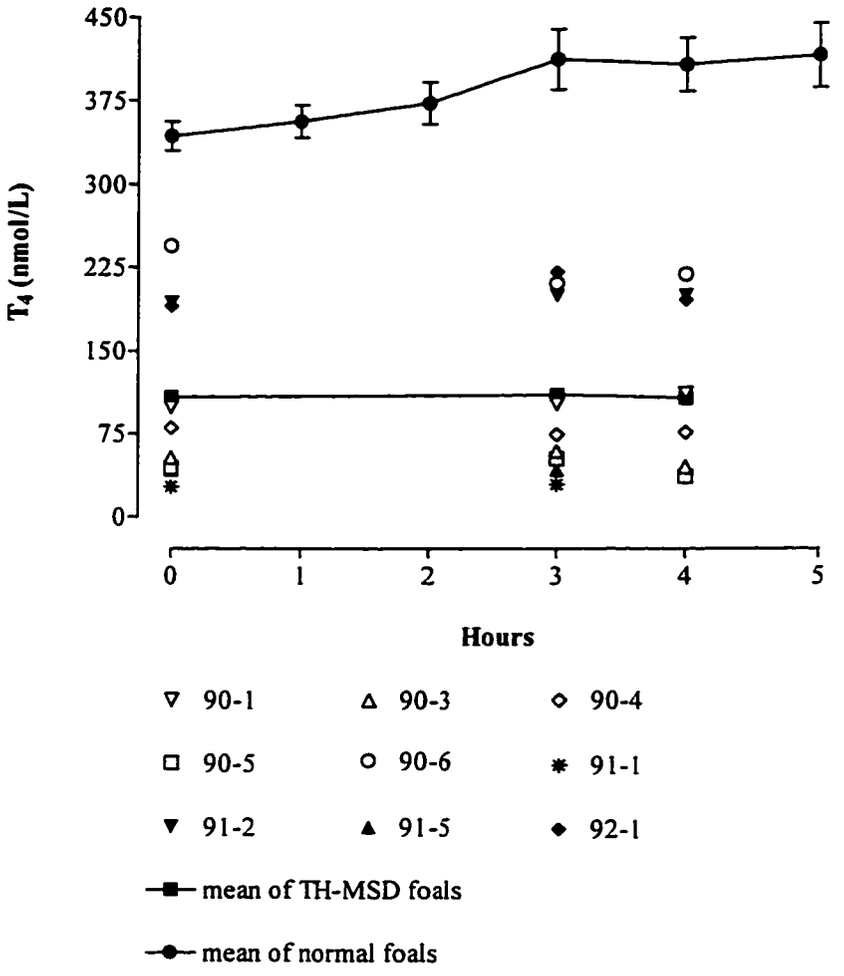

Figure 3.2. Serum thyroxine (T_4) concentrations in neonatal foals before and at various times after intravenous thyroid-stimulating hormone administration. The concentrations measured in 9 foals with thyroid gland hyperplasia and musculoskeletal deformities (TH-MSD) are presented individually and as mean values. The mean (± standard error) T_4 concentrations of 13 normally developed and healthy foals (see Figure 2.4) are presented for comparison. Mean values for TH-MSD and normal foals were significantly ($P < 0.0001$) different at 0, 3 and 4 hours.

Thyroid function of TH-MSD foals differed from that of normal foals in 2 major ways. Generally, TSH-MSD foals had lower baseline concentrations of T_3 and T_4 than normal foals, and affected foals failed to elevate either thyroid hormone in response to TSH administration.

3.4 Discussion

Twenty-five TH-MSD foals were recognized in the WCVM practice area during the 6 foaling seasons of 1990 through 1995. A review of the records of the WCVM Large Animal Clinic indicated that the TH-MSD syndrome was the most common problem of neonatal foals admitted to the WCVM during this time period.

Initially, these foals were a concern to their owners because of dystocia, stillbirth, weakness, failure to suckle, "crooked legs", or sepsis. Yet all were diagnosed with an underlying problem of congenital hypothyroidism and immaturity. The high rate of abnormal or difficult foaling (9 of 25 foals or 36%) reported in these TH-MSD foals is in marked contrast to the expected rate of dystocia reported in the literature. Although the rate of dystocia in mares is believed to vary with the breed, age, and parity of the mares, it has been estimated to be between 4 and 10%; light breed mares are thought to have a rate of < 5% (Youngquist 1986; Vandeplassche 1993). This information suggests that owners and veterinarians should consider the TH-MSD syndrome as the underlying cause of dystocia.

The most obvious signs of immaturity were those associated with the musculoskeletal system. However, as the author and attending clinicians gained

experience with affected foals, it became apparent that these congenitally hypothyroid foals often had an immature appearance due to a short, fine hair coat often described as "silky" in texture; and soft, pliable ears which failed to "stand" fully in some foals. When these various signs of immaturity are present in full term foals (i.e., ≥ 320 days) equine neonatologists refer to the foals as being dysmature and the condition as dysmaturity (Koterba 1990).

The investigations described herein represented a change in the method of research into TH-MSD foals from the descriptive nature of postmortem examination to the ante mortem recognition of affected foals. This allowed for the prospective testing of thyroid function of affected foals and led to the conclusion that TH-MSD foals were hypothyroid at birth and exhibited signs of incomplete development (i.e., immaturity) in spite of normal to often prolonged periods of gestation. As a consequence, the syndrome with which these foals were affected has come to be known as congenital hypothyroidism and dysmaturity (CHD) (Allen et al. 1993; Fretz 1994; Allen et al. 1996c). These studies demonstrated that CHD foals can be diagnosed ante mortem with a physical examination. Knowing the length of gestation and performing a radiographic examination of the carpal and tarsal bones will support the diagnosis and allow for an estimate of the severity of the immaturity. Thyroid function testing will also support the diagnosis but is not necessary since the pattern of lesions present in CHD foals is consistent and unique, and since every foal suspected to have CHD was confirmed to be hypothyroid when subjected to TSH response test.

Some final observations, but ones of importance for veterinarians and owners of CHD foals, concern the fate of affected foals. Only 1 of the 25 foals under study here was known to survive and have a productive life. Twenty-three foals died or were euthanatised for humane reasons. The major problems threatening the life of newborn CHD foals were failure of passive transfer of immunity and sepsis. This fact must receive equal consideration with the assessment of the degree of immaturity when offering a prognosis for survival, estimating the financial costs and nursing needs of treatment, and attempting to predict the future productivity of affected foals. Questions concerning these issues can best be addressed through prospective study with specific questions in place. This type of study will be optimized by the early identification and systematic evaluation of CHD foals.

4.0 FETAL DEVELOPMENT OF THE THYROID GLAND IN HORSES[1]

4.1 Introduction

Triiodothyronine (T_3) and thyroxine (T_4) levels in newborn foals are 10 to 20 times higher than in adult horses (see Appendices A, B, C, and D) and are greater than those reported in any other species in any other physiological state (Irvine and Evans 1975). This has led to speculation that the horse has been under considerable evolutionary selection pressure to produce these high levels at birth in order to develop and function in ways not required in other species for survival (Irvine and Evans 1975; Irvine 1984).

Little is known about the development of the thyroid gland during fetal life in the horse. In most species fetal plasma concentrations of T_3 are low and of T_4 high when compared with the neonate or adult (Fisher et al. 1973; Nathanielsz 1976; Fisher et al. 1977; Thomas and Nathanielsz 1983; Štrbák and Tomšík 1988; Diamond and Root 1991). Silver et al. (1991) found this to be true in the horse although they were working with small numbers of animals over a limited period late in gestation.

This investigation was conducted in preparation for the study to examine the effects of surgically-induced fetal hypothyroidism in horses, described in Chapter 5.0. The objectives of this investigation were (1) to document the morphological changes in

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[1] Portions of this chapter have been published (Allen et al. 1995).

the thyroid glands and (2) describe the temporal pattern of change of T_3 and T_4 in serum of equine fetuses during gestation.

4.2 Materials and Methods

Normal equine fetuses were obtained at a commercial abattoir within 30 minutes of the dams being stunned for slaughter. Fetuses were classified as normal based on gross external examination and visual examination of viscera during dissection. Blood was drawn from the fetal heart by syringe or evacuated glass tubes, allowed to clot in glass tubes placed in ice, and centrifuged for 10 minutes at 3,000 revolutions per minute (1,000 × *g*). Serum was removed from the clot using a plastic pipette, placed in a plastic vial with a water tight stopper, and frozen at -20°C in a non-frost-free freezer until assayed using a fluorescence polarization immunoassay (FPIA). Any hormone concentration which was less than the limit of detection of the assay was assigned a value that was the arithmetic mid-point between zero and the lowest detectable concentration. Therefore, T_3 and T_4 concentrations below the limit of detection of the assay were assigned values of 0.25 nmol/L and 2.5 nmol/L, respectively.

The thyroid glands were removed and placed in 10% neutral buffered formalin. After fixation, the right and left lobes were weighed on an electronic scale and the length, width and thickness measured with vernier calipers. The volume of each lobe was crudely estimated by multiplying the length, width and thickness. A correlation coefficient (r) was calculated as a measure of the strength and direction of all linear relationships between T_3 concentration, T_4 concentration, the combined weight of both

lobes of the thyroid gland after fixation, and the combined estimated volume of both lobes of the thyroid gland after fixation.

Fetuses were grouped by age after the fetal age in months was estimated using crown-to-rump length, weight, and external features of development (Roberts 1971; 1986). The mean T_3 and T_4 concentrations of each group were compared statistically by a Kruskal-Wallis nonparametric analysis of variance test. Where the results were considered significant ($P < 0.01$), a Dunn's post test was conducted to compare each group mean with the mean at 5 months gestation. All statistics were calculated with statistical software and a personal computer.

4.3 Results

All 66 fetuses examined were considered normal and used in this study. Several of the 12 fetuses classified as being 11 months gestation appeared to be fully developed and near parturition. Thyroid glands were difficult to identify and too small to handle in fetuses less than 4 months of gestation. Also, heart blood could not be obtained in fetuses less than 4 months of age. The mean weight, length, and estimated volume of each lobe of the thyroid gland after fixation are presented by group in Table 4.1. The mean weight, length and volume of the thyroid glands from equine fetuses increased markedly from 4 to 6 months of gestation; showed slight but consistent growth from months 6 to 10 of gestation; and increased markedly in weight and volume, and to a lesser degree in length, from months 10 to 11 of gestation.

Table 4.1. Mean weight, length and estimated volume of the right and left lobes of formalin-fixed thyroid glands from normally developed equine fetuses

	Right Lobe			Left Lobe		
Gestational Age (number of fetuses)	Weight (g)	Length (cm)	Volume (cm^3)	Weight (g)	Length (cm)	Volume (cm^3)
4 months (5)	< 0.50	1.00	0.37	< 0.50	1.03	0.48
5 months (11)	0.70	1.36	0.99	0.71	1.44	1.06
6 months (9)	2.54	2.22	4.12	2.43	2.37	4.04
7 months (12)	2.29	2.18	3.72	2.23	2.20	3.52
8 months (2)	3.05	2.45	4.93	3.20	2.55	5.05
9 months (3)	2.97	2.40	5.10	3.10	2.57	5.39
10 months (12)	2.84	2.43	4.67	2.98	2.48	4.91
11 months (12)	5.27	2.98	8.96	5.22	2.98	9.14

A summary of the correlation coefficients (r) is presented in Table 4.2. Not surprisingly, there was a very strong, positive, relationship between the volume and weight of the fetal thyroid glands, and between serum T_3 and T_4 levels. There was also a moderately strong, positive, relationship between the concentrations of thyroid hormones and the weight or volume of the fetal thyroid glands. The strength of the relationships between T_4 levels and the weight or volume of the thyroid glands were greater than those between T_3 and the weight or volume.

Serum concentrations of both T_3 and T_4 increased significantly ($P < 0.0001$) with fetal age (Figure 4.1). Levels of T_3 were undetectable (< 0.5 nmol/L) and of T_4 were low ($\bar{x}$ = 47.8 nmol/L) at 4 months gestation, and increased significantly ($P < 0.05$) in parallel fashion from 4 to 9 months. Peak levels ($\bar{x}$ ± standard error) of both T_3 and T_4 were observed in 9 month fetuses and equalled 2.6 (± 0.8) and 352.7 (± 12.7) nmol/L, respectively. High levels of both hormones persisted until 11 months gestation.

4.4 Discussion

The serum T_3 and T_4 concentrations of the 10 month old fetuses sampled in this study closely agreed with the concentrations reported by Silver et al. (1991) of 1.85 (± 0.31) and 316.60 (± 23.68) nmol/L, respectively, obtained from chronically catheterized pony fetuses between 290 and 310 days gestation. In the 11 month fetuses, T_3 levels were lower and the T_4 levels similar to those reported from neonatal foals (see Appendices C and D). This pattern is also similar to the one reported by Silver et al.

Table 4.2. The correlation between equine fetal serum triiodothyronine concentrations, serum thyroxine concentrations, the combined weights of both lobes of the thyroid gland after fixation, and the combined estimated volumes of both lobes of the thyroid gland after fixation

Variables	r[a]	*P*-value
volume and weight	0.9897	< 0.0001
T_3 and T_4	0.8126	< 0.0001
T_4 and weight	0.6723	< 0.0001
T_4 and volume	0.6592	< 0.0001
T_3 and weight	0.5842	< 0.0001
T_3 and volume	0.5659	< 0.0001

[a]r = correlation coefficient
T_3 = triiodothyronine; T_4 = thyroxine; volume = combined estimated volume of both lobes of the thyroid gland after fixation; weight = the combined weight of both lobes of the thyroid gland after fixation.

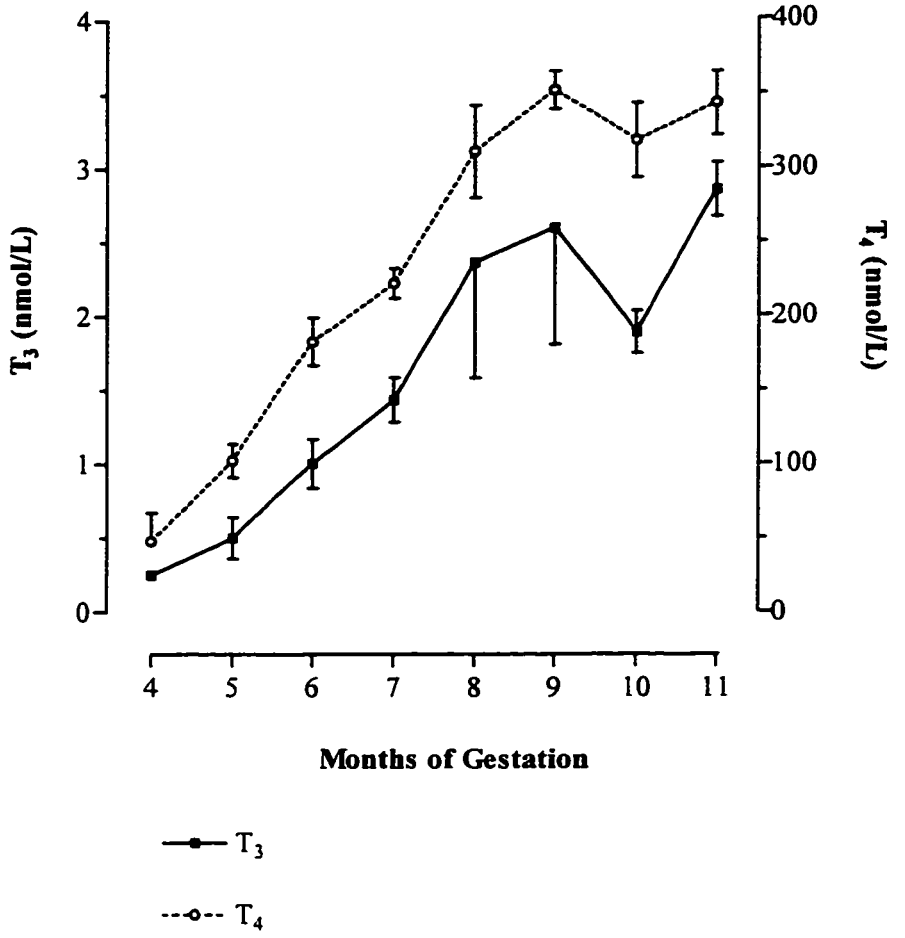

Figure 4.1. Mean (± standard error) concentrations of triiodothyronine (T_3) and thyroxine (T_4) in serum of equine fetuses between 4 and 11 months of gestation.

(1991) and appears typical of the other species studied. It also suggests that the stimulus for the rapid rise in T_3 levels of neonatal foals occurs very near parturition.

Finally, this study has indicated that interference of fetal thyroid function prior to 8 months of gestation will deprive the fetus of the effects of the highest concentrations of thyroid hormone for about 100 days or approximately the last 30% of gestation. This information is important when considering a model of fetal hypothyroidism in the horse. This study has further demonstrated that the thyroid gland lobes of the 8 month fetus would be large enough (2.5 cm long and 3.13 g) to be handled by a surgeon attempting to perform a fetal thyroidectomy.

5.0 SURGICALLY-INDUCED HYPOTHYROIDISM IN THE EQUINE FETUS[1]

5.1 Introduction

It was demonstrated in Chapter 3.0 that foals with the syndrome of thyroid gland hyperplasia and musculoskeletal deformities (TH-MSD) were congenitally hypothyroid and dysmature (CHD). However, it remains unclear whether all the anomalies present in CHD foals are caused concurrently by an as yet unidentified agent, or if affected foals are primarily hypothyroid in utero with the associated lesions being the result of the hypothyroid state.

The objectives of this investigation were (1) to have pregnant mares carry their foals to term following partial thyroidectomy or sham surgery performed at about 215 days of gestation; (2) to document the effects of partial thyroidectomy on the growth and development of the equine fetus; and, (3) to compare the anomalies, if any, present in the newborn, partially thyroidectomized foals with those reported in the spontaneously occurring CHD foals seen in western Canada.

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[1] Portions of this chapter have been accepted for publication (Allen et al. 1996a).

5.2 Materials and Methods

5.2.1 Animals and animal management

Twenty-five, light breed mares between 2 and 7 years of age were kept on native grass pasture during the months of May through October. In addition to the native grass, the mares were fed mixed alfalfa and grass hay at a rate of 1 to 2% of their body weight per day. They also had free access to a granular vitamin and mineral supplement, blocks of sodium chloride salt with added iodine and cobalt, and well water which had been tested and found fit for human consumption. The mares were bred naturally on pasture by an Arabian stallion. Dates of conception were estimated by observing the mares for behavioral evidence of estrus, combined with manual and ultrasonographic transrectal examination of the reproductive tract. From November until foaling, the mares were kept in a pen provided by the University of Saskatchewan Animal Care Unit located at the Western College of Veterinary Medicine (WCVM). In the pen, the mares had free access to mixed alfalfa and grass hay, vitamins and minerals as described above, and water supplied to the City of Saskatoon.

Mares were administered about 200 μg/kg of ivermectin orally in May, July, October and March; vaccinated in May against equine influenza virus (strain A1 and multiple strains of A2), equine herpesvirus (strains 1 and 4), Eastern and Western equine encephalitis viruses, along with tetanus toxoid, and rabies virus in May; and vaccinated against equine herpesvirus (strains 1p and 1b) at about the 5th, 7th, and 9th months of gestation. All mares had a normal response to a thyroid-stimulating hormone (TSH^b) response test (Held and Oliver 1984; Chapter 2) conducted in June.

5.1.2 Surgery

Between 202 and 238 days of gestation, 21 mares and their fetuses underwent surgery to expose the fetal thyroid gland. In 14 instances as much fetal thyroid gland as possible was removed while the other 7 fetuses served as sham-operated control animals. Six of 21 mares carried their foals to term (i.e., at least 320 days gestation) following surgery and their management is described.

The 6 mares contributing foals to this study (M10, M13, M14, M17, M18, and M19) had similar management around the time of surgery. They were admitted to the Veterinary Teaching Hospital of the WCVM, 48 to 72 hours before surgery and hay was withheld for 12 to 18 hours prior to the induction of general anesthesia. A combination of antibiotics were administered prophylactically. All mares received about 30 mg/kg of trimethoprim and sulfamethoxazole in a fixed 1:5 ratio, orally, b.i.d., starting 12 hours prior to surgery and continuing for 6 to 10 days after surgery, except for M14 which received it for 21 days after surgery; and 20,000 to 40,000 IU/kg of sodium penicillin G, intravenously, 1 hour prior to surgery and 20,000 IU/kg of procaine penicillin G, intramuscularly, b.i.d., for 6 to 7 days after surgery. The mares also received 1.1 mg/kg of flunixin meglamine, intravenously, at 12 hours and 1 hour prior to surgery, and 0.6 mg/kg, intramuscularly, b.i.d., for 3 to 4 days following surgery. Three mares (M13, M14, and M19) also received 43 mg of altrenogest, orally, the day before and the day of surgery, and 21.5 to 43 mg, orally, daily for 3 or 4 days after surgery. Mare M14 also received 300 to 400 μg of clenbuterol hydrochloride, intravenously or orally, b.i.d., on 7 of the first 11 days following surgery. The ventral abdomen of each mare was clipped

and antiseptically prepared with water, 4% chlorhexidine gluconate, and 70% isopropyl alcohol prior to surgery.

Prior to the induction of anesthesia, the 6 mares were premedicated with 0.5 mg/kg of xylazine, intravenously, followed by 5% glyceryl guaiacolate in a 5% dextrose solution (compounded at the WCVM Pharmacy), intravenously, until they appeared relaxed. General anesthesia was induced with 2 mg/kg of ketamine hydrochloride administered intravenously and was maintained on halothane vaporized in oxygen using an out-of-circle ventilator for large animals and delivered through an endotracheal tube with positive pressure ventilation. All mares received dobutamine hydrochloride, intravenously, to effect, and all but M13 received 20 to 30 mg of butorphanol, intravenously, during surgery.

The mares were placed in dorsal recumbency and prepared for a midline laparotomy. A 30 cm midline incision was made from 10 cm cranial to the umbilicus to the cranial limit of the mammary glands to expose the abdominal viscera. The fetus was manipulated within the uterus to move the head and ventral aspect of the neck into a superficial position. The uterus was then carefully elevated into the incision of the abdominal wall. Avoiding large vessels, incisions were made into the uterus, chorioallantois, amnion and fetal skin. Blunt dissection of the neck was used to expose the fetal thyroid gland. The fetuses of mares M10, M13, M17, and M19 had as much of the thyroid gland removed as possible. The fetuses of mares M10, M17 and M18 received 120 mg of trimethoprim and 600 mg of sulphadoxine, intramuscularly, intraoperatively. The fetus of mare M13 received 2 g of ampicillin, intravenously, and

4 g of ampicillin plus 3.2 g of trimethoprim and 16 g of sulphadiazine infused into the amniotic and allantoic fluids, intraoperatively. The fetus of mare M14 received 4 g of ampicillin intravenously, and 10 g of ampicillin plus 2.4 g trimethoprim and 12 g sulphadiazine infused into the amniotic and allantoic fluids intraoperatively. The fetus of mare M19 received 160 mg of trimethoprim and 800 mg of sulphadiazine intramuscularly and 10 g of ampicillin plus 3.2 g of trimethoprim and 16 g of sulphadiazine infused into the amniotic and allantoic fluids.

The fetal skin and the amnion were each closed with 4-0 monofilament polydioxanone suture in a simple continuous pattern. The uterus was closed with 0 monofilament polydioxanone suture in a 2 layer (oversewn) Utrecht pattern taking care not to include the chorioallantois. The linea alba was closed with #2 monofilament polydioxanone suture in an interrupted cruciate pattern. The subcutaneous tissues were apposed with 00 monofilament polydioxanone suture in a simple continuous pattern, and the skin was closed using stainless steel staples. Following recovery from anesthesia, the 6 mares were returned to stalls in the Veterinary Teaching Hospital and were returned to the outdoor pen between 7 and 14 days after surgery without further therapy, except for mare M14 which received trimethoprim and sulfamethoxazole, orally, b.i.d., for an additional 7 days while kept outdoors.

5.2.3 Data collection

Serum was harvested from blood samples taken from each of the 6 foals delivered after 320 days gestation. A physical examination was performed and a TSH[b]

response test administered (Shaftoe et al. 1988) on the 5 foals born alive. Live foals were euthanàtised within 12 hours of birth with a lethal dose of sodium pentobarbital and a postmortem examination conducted immediately. Serum samples were analysed for T_3 and T_4 levels using the fluorescence polarization immunoassay (FPIA) described in Chapter 2.0.

Characteristics of immaturity that were considered included weakness and inability to stand; a short, fine, "silky" hair coat; soft, pliable ears; laxity of the joints and tendons of the distal legs; and incomplete ossification of the bones of the limbs. The presence of a short, fine hair coat was determined subjectively and by noting if the hair on the hind feet was fully developed and long enough to cover the coronary bands. Radiographs were made of the right fore and right hind legs and all legs were examined grossly. The degree of skeletal development of sham-operated control foals was assessed by comparing the pattern of ossification in the bones of the limbs with that expected in normally developed foals (Getty and Hillmann 1975) and through the use of the grading system modified from Adams and Poulos (1987, 1988) and Adams (1990) which was presented in Table 3.1. The degree of skeletal development of partially thyroidectomized foals was compared to sham-operated control foals. Dysmaturity was defined as the presence of signs of immaturity in a full term foal (Koterba 1990). Thyroid glands or thyroid gland remnants were fixed in 10% neutral buffered formalin and weighed on an electronic scale. Portions of the thyroid glands or the thyroid gland remnants were then embedded in paraffin, prepared routinely, sectioned at 4 to 6 μm, and stained with hematoxylin and eosin and with the periodic acid-Schiff reaction.

The median, as a crude measure of central tendency, was calculated for continuous variables. Correlation coefficients were calculated for the relationships between each of the thyroid hormones and the weight of the thyroid gland or thyroid gland remnant. Additional statistical analyses were not appropriate.

5.3 Results

Two sham-operated control foals (F14 and F18) and 4 thyroidectomized foals (F10, F13, F17 and F19) were carried to term. Foal F10 was stillborn; parturition of foal F17 was induced with 2 doses of 20 IU of oxytocin, administered intravenously, about 1 hour apart; and foals F13, F14, F18, F19 were delivered spontaneously and without assistance. Fifteen of 21 (71.4%) mares aborted between 3 and 74 days after surgery. Postmortem examination of the fetuses and placentas combined with statistical analysis of potential risk factors identified the introduction of bacteria during surgery as the likely cause of 6 of the 15 (40.0%) abortions. Complications of anesthesia such as hypotension and hypercapnia were possible causes of 5 (33.3%) abortions and 1 abortion (6.7%) was associated with each of an ascending bacterial placentitis, hydrocephalus with hydramnios, and lymphocytic myocarditis. One abortus was not examined. Abortions due to bacterial infection were eliminated as experience was gained both in the perioperative management of the mares and in the surgical procedures.

Details regarding the sex, weight, size, and features of gestation of the foals contributing data to this study are presented in Tables 5.1.1 and 5.1.2. The 2 groups of

Table 5.1.1. Selected features of the history and signalment of sham-operated control foals

	Foal		
Feature	F18	F14	Median
Sex	female	female	-
Weight (kg)	32.5	37.2	34.85
Crown-to-rump length (cm)	95	100	97.5
Length of gestation (days)	336	331	333.5
Gestational age at surgery (days)	204	216	210
Days from surgery to birth (days)	132	115	123.5
Proportion of gestation after surgery (%)	39.3	34.7	37.0

Table 5.1.2. Selected features of the history and signalment of partially thyroidectomized foals

Feature	Foal				Median
	F10	F17	F19	F13	
Sex	female	male	male	female	-
Weight (kg)	29.1	36.0	36.8	25.1	32.6
Crown-to-rump length	94	96	100	95	95.5
Length of gestation (days)	337[a]	358[b]	344	345	344.5
Gestational age at surgery (days)	202	209	208	238	208.5
Days from surgery to birth	135	149	136	107	135.5
Proportion of gestation after surgery (%)	40.1	41.6	39.5	31.0	39.8

[a] = foal was stillborn; [b] = parturition was induced.

foals did not appear to differ in terms of their weight or crown-to-rump length. However, each of the partially thyroidectomized foals had a longer gestation than either of the sham-operated control foals.

Findings of the physical and postmortem examinations are presented in Tables 5.2.1 and 5.2.2. Sham-operated control foals behaved normally and had normal physical development. In contrast, the partially thyroidectomized foals had abnormal locomotor skills and mentation, as well as 1 to 5 signs of physical immaturity. Examples of differences in the development of the hair coat are presented in Figure 5.1 and of differences in ossification of the carpal and tarsal bones in Figure 5.2. Based on these findings each of the 4 partially thyroidectomized foals was considered dysmature.

Sham-operated control foals consistently exhibited the pattern of ossification that was expected in normally developed full term foals. In contrast, partially thyroidectomized foals had numerous and marked deficiencies in their skeletal development (see Appendices E, F, G, and H).

Indicators of thyroid gland morphology and function were measured and are presented in Tables 5.3.1 and 5.3.2. The weights of the fixed thyroid glands of sham-operated control foals were 2.48 to 14.25 times the weight of the fixed thyroid gland remnants of the partially thyroidectomized foals (Figure 5.3). Histologically, the thyroid glands of sham-operated control foals were normal, with round to ovoid follicles which were moderately variable in size and composed of a simple, cuboidal to short columnar epithelium, and with abundant, deeply staining, homogeneous colloid. The thyroid gland remnants of partially thyroidectomized foals were hyperplastic. Follicles were

Table 5.2.1. Selected features of the physical and postmortem examination of sham-operated control foals

Feature	Foal		
	F18	F14	Median
Mentation	normal	normal	-
Locomotor skills	St,F,N	St,F,N	-
Short, fine ("silky") hair coat	no	no	-
Soft, pliable ears	no	no	-
Joint and tendon laxity	no	no	-
Degree of mandibular prognathism (mm)	1 to 2	1 to 2	1.5
Size of defect around umbilicus (cm)	4	3.5	3.75
Flexural deformity of the legs	mild[a]	no	-
Lesions of the CDET	no	no	-
Development of the carpal and tarsal bones (Grade[b])	4	3	3.5
Dysmaturity	no	no	-

CDET = common digital extensor tendons
St = stand without assistance; F = able to follow mare; N = attempt to nurse from mare
[a] = carpal area of right front leg only
[b] = criteria are presented in Table 3.1

Table 5.2.2 Selected features of the physical and postmortem examination of partially thyroidectomized foals

Feature	Foal				Median
	F10	F17	F19	F13	
Mentation	NA	D	D	D	-
Locomotor skills	NA	Lr	Sr	Sr,StA	-
Short, fine ("silky") hair coat	yes	yes	yes	no	-
Soft, pliable ears	yes	yes	yes	no	-
Joint and tendon laxity	marked	marked	marked	no	-
Degree of mandibular prognathism (mm)	6	12	7	0	6.5
Size of defect around umbilicus (cm)	8	4.5	8	1	6.3
Flexural deformity of the legs	no	no	no	no	-
Lesions of the CDET	no	no	H,T,R	H,T	-

Table 5.2.2. (continued)

Selected features of the physical and postmortem examination of partially thyroidectomized foals

Feature	Foal				Median
	F10	F17	F19	F13	
Development of the carpal and tarsal bones (Grade[a])	0	0	1	2	0.5
Dysmaturity	yes	yes	yes	yes	-

CDETs = common digital extensor tendons
NA = not applicable; D = depressed and lethargic
Lr = lateral recumbency; Sr = sternal recumbency; StA = stand with assistance
H = hemorrhage; T = partial tearing; R = complete rupture
[a] = criteria are presented in Table 3.1.

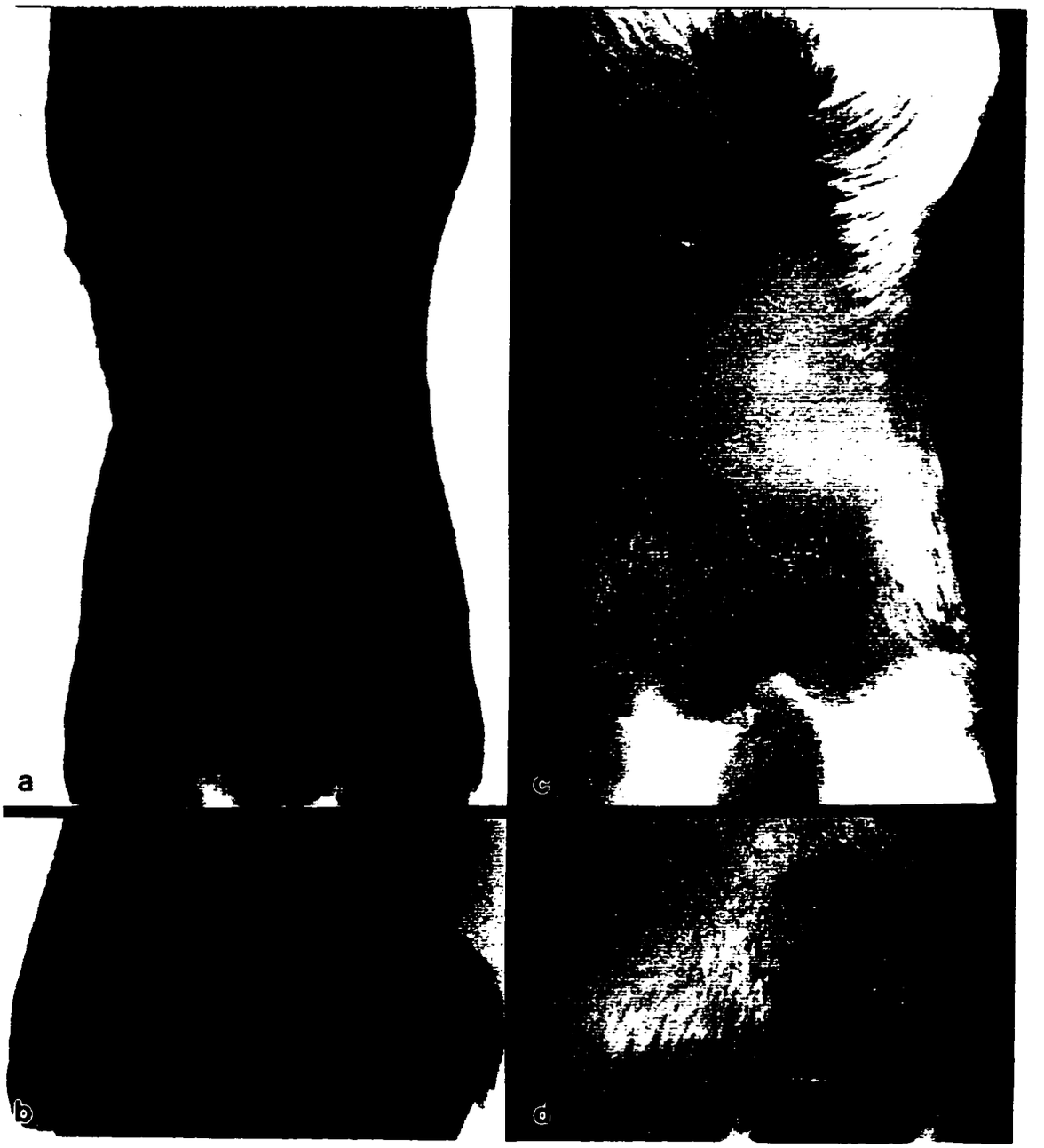


Figure 5.1. The plantar (a and c) and medial (b and d) surfaces of the distal right hind leg of foals F18 (a and b) and F10 (c and d). There is normally developed, coarse, long hair completely covering the pastern area and coronary band of sham-operated control foal F18. The hair on the pastern area of partially thyroidectomized foal F10 is not fully developed, is fine, incompletely covers the plantar surface, and is too short to hide the coronary band.

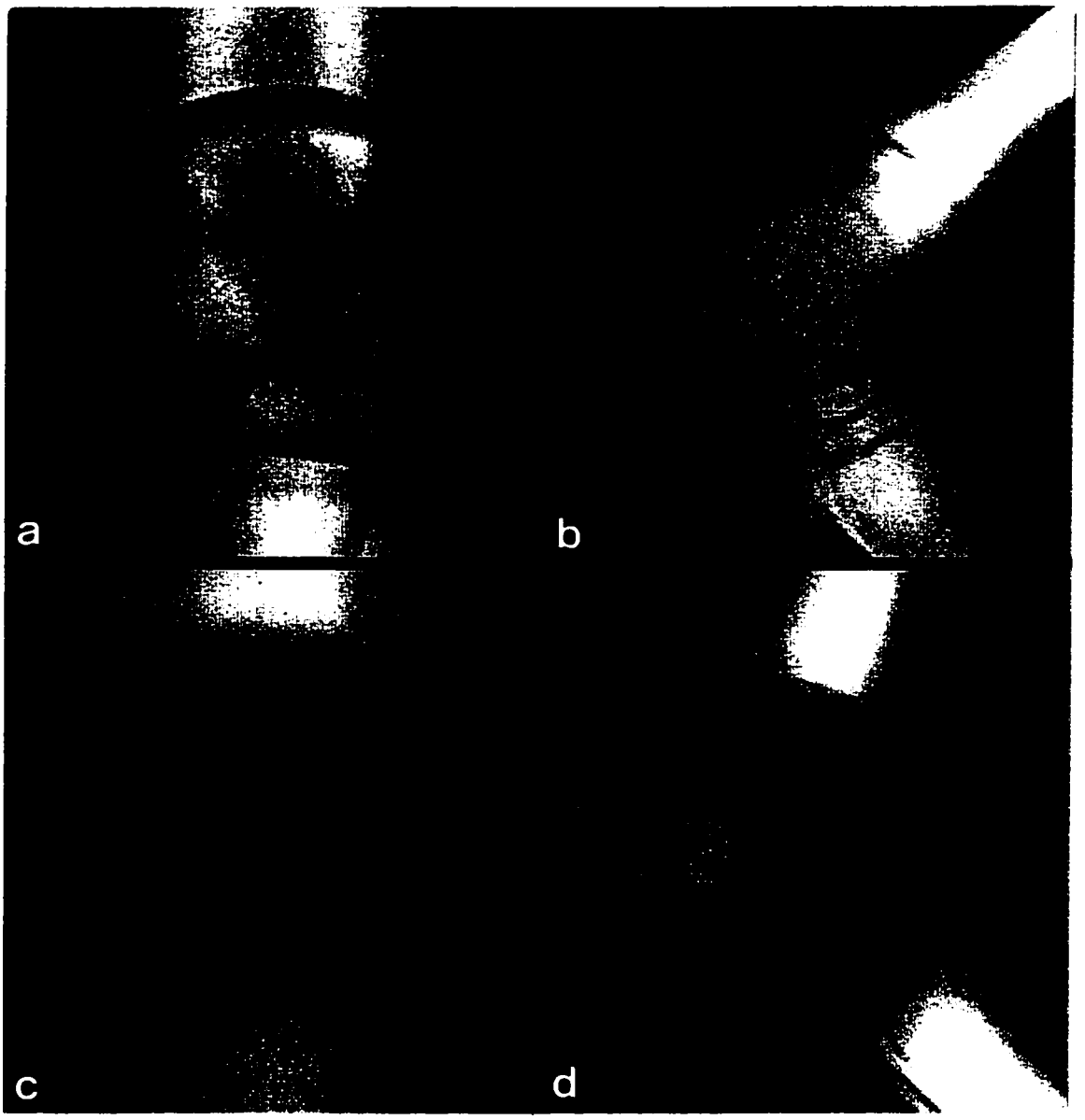


Figure 5.2. Dorsopalmar radiographs of the right carpus from foals F14 (a) and F17 (c) and lateromedial radiographs of the right tarsus from foals F18 (b) and F10 (d). Radiographs were interpreted using the criteria in Table 3.1. The carpal and tarsal bones of sham-operated control foals F14 and F18 were well ossified, normal for full term foals, and consistent with Grades of 3 and 4, respectively. There was an absence of ossification in the carpal and tarsal bones of partially thyroidectomized foals F17 and F10, which is abnormal in full term foals, and consistent with a Grade of 0.

Table 5.3.1 Selected features of thyroid gland morphology and function of sham-operated control foals

Feature	Foal		Median
	F18	F14	
Total fixed[a] weight (g)	11.4	8.2	9.8
Histologic diagnosis	normal	normal	-
Resting T_3 level (nmol/L)	7.60	4.01	5.81
Peak[b] T_3 level (nmol/L)	16.90	11.26	14.08
Increase[c] in T_3 (%)	122	181	151.5
Resting T_4 level (nmol/L)	364	261	312.5
Peak[b] T_4 level (nmol/L)	376	269	322.5
Increase[c] in T_4 (%)	3.3	3.1	3.2

T_3 = triiodothyronine; T_4 = thyroxine
[a] = fixed in 10% neutral buffered formalin
[b] = maximum level obtained during thyroid stimulating hormone response test (see Figures 5.1 and 5.2)
[c] = increase from resting level to maximum level during thyroid stimulating hormone response test expressed as a percentage (see Figures 5.1 and 5.2).

Table 5.3.2 Selected features of thyroid gland morphology and function of partially thyroidectomized foals

Feature	Foal				Median
	F10	F17	F19	F13	
Total fixed[a] weight (g)	0.8	1.0	2.6	3.3	1.8
Histologic diagnosis	H,C	H,C	H,C	H,C	-
Resting T_3 level (nmol/L)	0.92	0.86	1.55	3.92	1.2
Peak[b] T_3 level (nmol/L)	NA	1.01	1.75	7.79	1.8
Increase[c] in T_3 (%)	NA	17	13	99	17
Resting T_4 level (nmol/L)	26	35	82	243	58.5
Peak[b] T_4 level (nmol/L)	NA	29	76	270	76
Increase[c] in T_4 level (%)	NA	-17	-7	11	-7

Table 5.3.2 (continued)

T_3 = triiodothyronine; T_4 = thyroxine; H = hyperplasia; C = abnormal colloid; NA = not available

[a] = fixed in 10% neutral buffered formalin

[b] = maximum level obtained during thyroid stimulating hormone response test (see Figures 5.1 and 5.2)

[c] = increase from resting level to maximum level during thyroid stimulating hormone response test expressed as a percentage (see Figures 5.1 and 5.2)

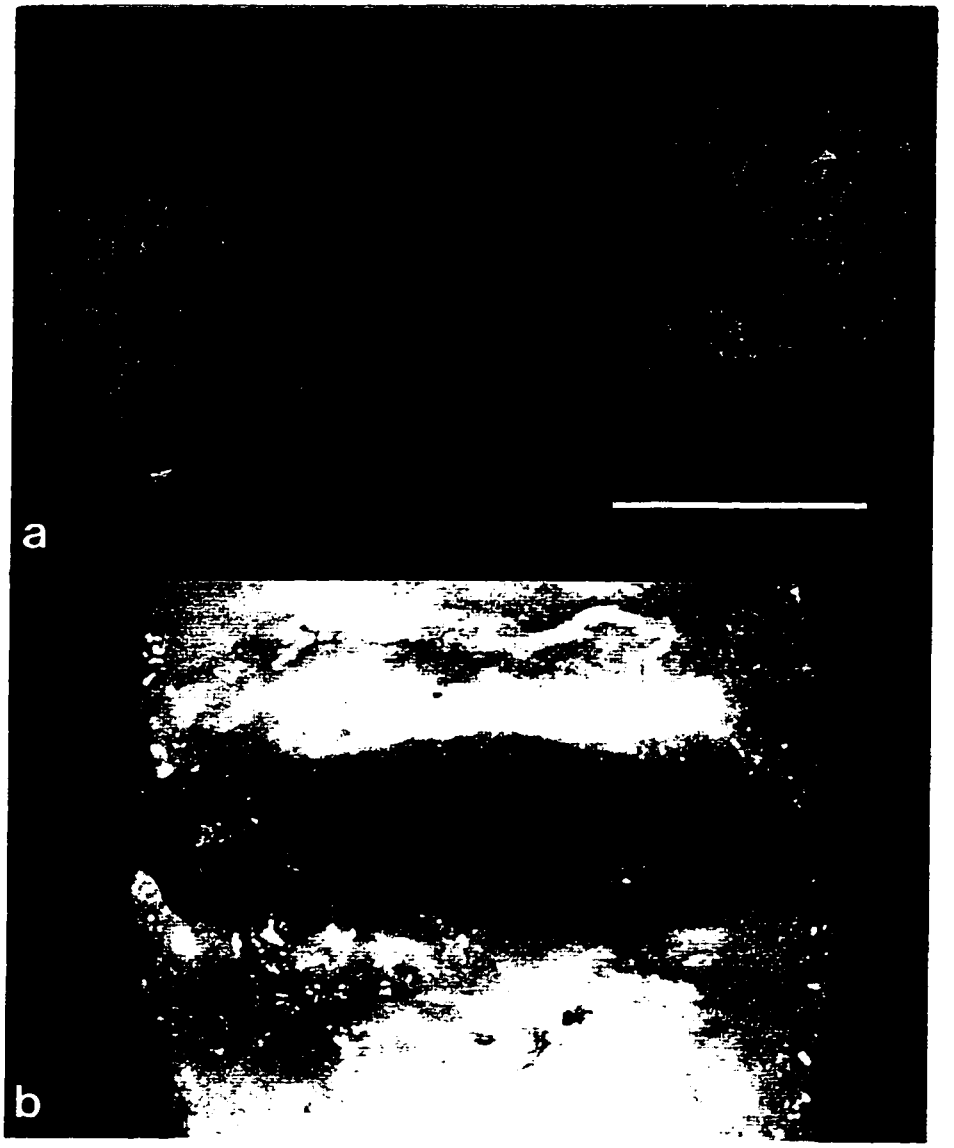


Figure 5.3. The fixed thyroid gland of foal F18 (a) and fixed thyroid gland remnant and trachea of foal F10 (b). The thyroid gland from sham-operated control foal F18 is normally developed with 2 lobes and a thin isthmus of fibrous tissue (scale = 2 cm; fixed weight = 11.4 g). The thyroid gland remnant of foal F10 represents hypertrophy and hyperplasia of the follicular epithelium in the isthmus following surgical removal at 202 days gestation of both lobes of the thyroid gland. The remnant was about 2 cm in length and 0.5 cm in diameter (fixed weight = 0.8 g).

markedly variable in size, irregularly shaped, angular or collapsed, and composed of short to tall columnar epithelium; the epithelium was sometimes multiple layers thick. Normal colloid was scant or absent; instead the majority of follicles contained a poorly staining, granular to vesicular material (Figure 5.4). The complete results of TSH response tests are presented in Figures 5.5 and 5.6. Since foal F10 was stillborn, only resting or baseline values of T_3 and T_4 were determined. Also, because foal F17 died prior to 3 hours after TSH administration, the last serum sample was collected at 2.5 hours. The resting T_3 levels of the 2 sham-operated control foals were 1.02 to 8.84 times higher than that of the 4 partially thyroidectomized foals. The 2 sham-operated control foals showed an elevation in their T_3 levels by 122% and 181% following TSH administration. In contrast, none of partially thyroidectomized foals demonstrated an increase in T_3 levels of as much as 100% and 2 foals could not increase their T_3 levels more than 17%. The resting T_4 levels of the 2 sham-operated control foals were 1.07 to 14 times higher than that of the 4 partially thyroidectomized foals. None of the foals was able to elevate their T_4 levels by an appreciable amount following TSH administration.

There was a very strong, positive, relationship between each of the thyroid hormones and the weight of the thyroid gland or thyroid gland remnant. Specifically, the correlation coefficient for the relationship between T_3 and thyroid weight was 0.9415 ($P = 0.005$), and for the relationship between T_4 and thyroid weight it was 0.9183 ($P = 0.0097$).

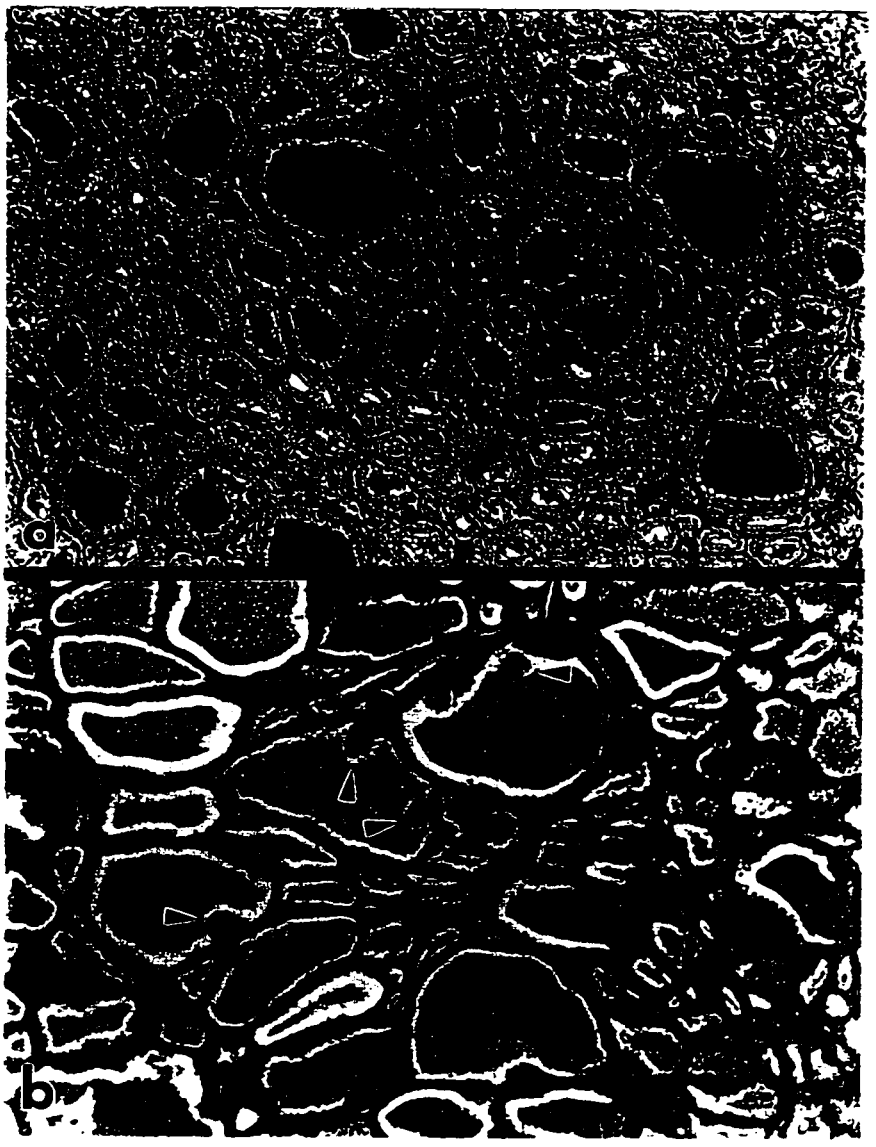
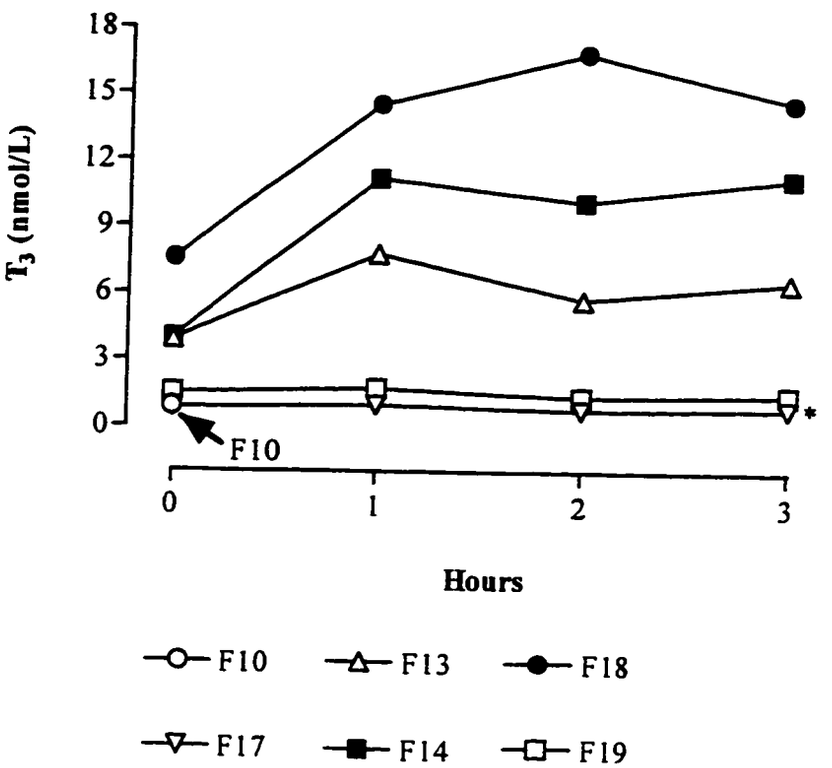
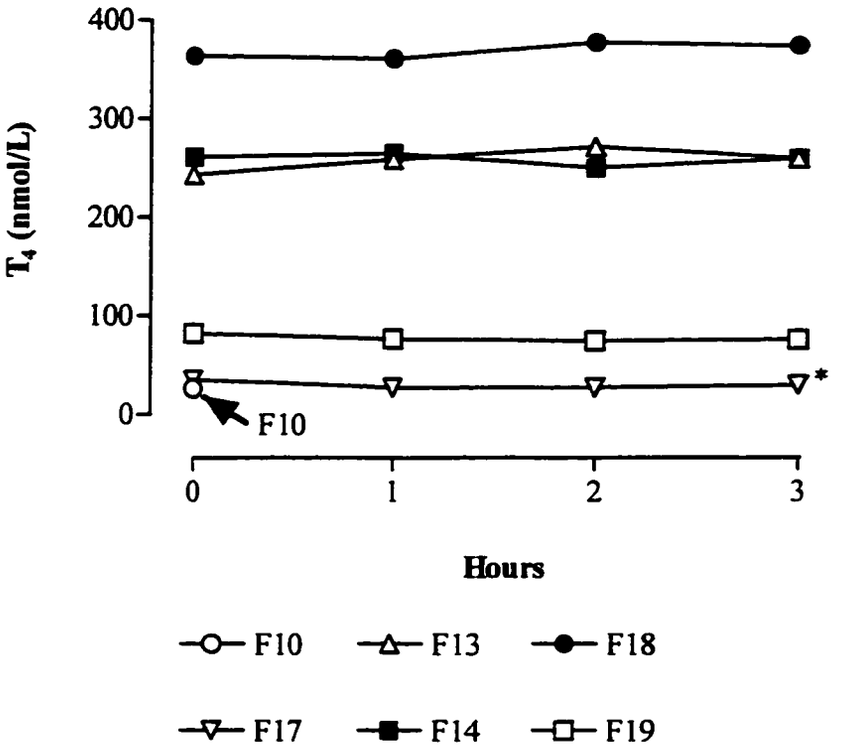


Figure 5.4. Photomicrograph of the thyroid gland of foal F18 (a) and of the thyroid gland remnant of foal F17 (b) about 3 hours after thyroid stimulating hormone administration. The thyroid gland of sham-operated control foal F18 is normally developed, with round to ovoid follicles composed of a single layer of cuboidal epithelium, and abundant, deeply staining (black), colloid. The thyroid gland remnant of partially thyroidectomized foal F17 contains hyperplastic thyroid tissue: follicles are irregularly shaped, angular, lined by short to tall columnar epithelium which is multiple layers thick in many areas (arrow heads), and the majority are filled with poorly staining, granular to vesicular material with absence of normal colloid. Periodic acid-Schiff reaction; original magnification × 25; scale bar = 100 μm.



*actual time for F17 was 2.5 hours

Figure 5.5. Serum triiodothyronine (T_3) concentrations in sham-operated control foals (F14 and F18) and partially thyroidectomized foals (F10, F13, F17, and F19) before and for 3 hours after thyroid-stimulating hormone administration.



*actual time for F17 was 2.5 hours

Figure 5.6. Serum thyroxine (T_4) concentrations in sham-operated control foals (F14 and F18) and partially thyroidectomized foals (F10, F13, F17, and F19) before and for 3 hours after thyroid-stimulating hormone administration.

5.4 Discussion

Screening programs for the detection of congenital hypothyroidism in human infants has been established in developed countries for many years. While there is variation owing to geography or ethnicity, sex, method of screening, and other technical aspects of the tests used, the incidence of congenital hypothyroidism is generally described as 1:3500 to 1:4500 births (Grant and Smith 1988; Letarte and Garagorri 1989; Behrman 1992; Lorey and Cunningham 1992; Toublanc 1992). Some of the features of congenital hypothyroidism in infants include prolonged gestation (in 20 to 50% of cases); umbilical hernia (25 to 60%); lethargy (30 to 40%); hypotonic muscles (35%); and retarded skeletal development (40 to 75%) (LaFranchi 1979; Letarte and Garagorri 1989; Newland et al. 1991; Behrman 1992; Grant et al. 1992; Styne 1994). It has been suggested that the delay in skeletal maturation at birth is proportional to the severity and duration of the fetal hypothyroidism, and hence the amount of functioning thyroid tissue (Ilicki et al. 1990; Newland et al. 1991; Grant et al. 1992). If the hypothyroidism continues untreated, bone maturation is reported to nearly stop, the physeal growth plates may remain open indefinitely, and the radiographic appearance of the bones of affected individuals is so distinctive it is considered by some to be virtually diagnostic (DeLellis 1989; Chew 1991). These congenitally hypothyroid neonates not only exhibit delayed ossification of epiphyses, but irregular and fragmented epiphyseal ossification referred to as epiphyseal dysgenesis (Wilkins 1941; LaFranchi 1979; DeLellis 1989; Chew 1991; Newland et al. 1991). Further, the medullary cavities

in the tubular and flat bones are characteristically small and narrow, with corresponding thickening of the overlying cortex (Silverman 1993).

Congenital hypothyroidism has been described in altricial domestic mammals like the rat (Scow and Simpson 1945; Becks et al. 1948; Goldberg and Chaikoff 1949; Noback et al. 1949; Weiss and Noback 1949; Becks et al. 1950; Ray et al. 1950, 1954; Hamburgh and Lynn 1964; Shrader et al. 1979), cat (Arnold et al. 1984; Sjollema et al. 1991; Tanase et al. 1991; Jones et al. 1992), dog (Chastain et al. 1983; Greco et al. 1985; Medleau et al. 1985; Robinson et al. 1988; Zerbe et al. 1988; Greco et al. 1991; Saunders and Jezyk 1991; Mooney and Anderson 1995), and rhesus monkey (Lusted et al. 1953; Kerr et al. 1972; Olson et al. 1985). In these species, congenitally hypothyroid individuals appear normal at birth, but develop signs of hypothyroidism during their first several days to several weeks of life. Typically, affected individuals are weak; lethargic and depressed; constipated with a distended abdomen; fail to grow, and develop a dwarfish appearance including a large broad head, small ears, large tongue, as well as a short neck and limbs. They retain their dense, fluffy coats and fail to grow adult guard hairs; and they fail to shed deciduous teeth. Radiographic examination reveals failure of skeletal maturation, characterized by shortened vertebral bodies, delayed physeal closure, an absence or delay of epiphyseal ossification, and epiphyseal dysgenesis which has been described as pathognomonic for congenital hypothyroidism in dogs (Chastain 1990). Congenitally hypothyroid rhesus monkeys also experienced prolonged gestations (Olson et al. 1985).

In the sheep, a precocious domestic mammal, congenitally hypothyroid individuals are abnormal at birth. Typically, congenitally hypothyroid lambs are born after a prolonged gestation; are small or stunted; hairless or have a sparse covering of coarse hair; depressed, lethargic, weak and unable to stand; constipated; and usually die within a few days after birth. Congenitally hypothyroid lambs also have short bones which, radiographically, have retarded maturation in the form of delay in the appearance of ossification centers. When ossification is present within epiphyses and other small bones, it is irregular and fragmented, i.e., dysgenic (Ferguson et al. 1956; Lascelles and Setchell 1959; Setchell et al. 1960; Hopkins and Thorburn 1972; Thorburn and Hopkins 1973; Chapman et al. 1974). Hopkins and Thorburn (1972; Thorburn and Hopkins 1973) have suggested that the lack of osseous maturity present at birth in congenitally hypothyroid lambs is related to the duration of in utero hypothyroidism. The congenitally hypothyroid lambs also had abnormally shaped bones with a poorly hollowed medullary cavity (Lascelles and Setchell 1959), which is believed to be the result of a failure to remodel primary bone (Hopkins and Thorburn 1972).

A description of congenitally hypothyroid Angora goats, another precocious species, also indicated that kids were abnormal at birth. At several weeks of age, congenitally hypothyroid animals were dull; stunted; and had a short, broad head and mandibular prognathism (Bath et al. 1979).

This study has provided evidence of the effects of in utero hypothyroidism leading to congenital hypothyroidism in foals. Congenitally hypothyroid foals in this study had prolonged gestations, were depressed, were weak and unable to stand, had

umbilical hernias, and had an incomplete coat that tended to be short and immature in texture. Most striking was the delay in skeletal maturation characterized by the retarded ossification of epiphyses, cuboidal bones of the carpi and tarsi, patellae, proximal sesamoid bones, and distal sesamoid (navicular) bones; and by delayed closure of physes. As with human infants and surgically thyroidectomized lambs, there appears to be a relationship between the severity of fetal hypothyroidism - estimated by weight of the thyroid gland or thyroid gland remnants and the resting T_3 and T_4 levels - and the degree of maturation of the bones of the limbs in these foals.

The congenitally hypothyroid foals in this study also had mandibular prognathism. The pathogenesis of this lesion is not known, but may represent a failure of the maxilla to lengthen and advance rostrally due to retarded growth at the synchondroses between the occipital and basisphenoid bones (sphenooccipital joint) and the basisphenoid and presphenoid bones (intersphenoid joint) (Sisson 1975).

Two of the partially thyroidectomized foals in this study had hemorrhage, tearing and rupture of the common digital extensor tendons. Again, the pathogenesis of this lesion in not known. However, it is interesting to note that these foals were the 2 congenitally hypothyroid foals strong enough to attempt to stand, suggesting that the tendons of congenitally hypothyroid foals are not as strong as normally developed foals, and that a certain amount of stress is required to bring about injury. It appears that congenitally hypothyroid foals that do not stress their tendons will not injure them. This hypothesis could be tested by comparing the development and tensile strength of tendons from congenitally hypothyroid foals and normal foals.

The anomalies and lesions present in the partially thyroidectomized foals of this study are comparable to congenitally hypothyroid neonates of other species including human infants. The histories, clinical appearances, and postmortem lesions of the congenitally hypothyroid foals is also strikingly similar to that reported in the CHD foals described previously, including the lack of remodelling, reduction in size of the bone marrow cavity, and thick cortices of the long bones, i.e., osteopetrosis (Allen 1995).

Flexural deformities of the legs were not seen in the congenitally hypothyroid foals of this study, but were common lesions in CHD foals. The reason for this is not known. Perhaps, it relates to the duration of fetal hypothyroidism. The congenitally hypothyroid foals in this study were partially thyroidectomized for 107 to 149 days. The studies described in Chapter 4 (also Allen et al. 1995) suggested that the equine fetal thyroid gland becomes active by the 4th or 5th month of gestation, which is on average, about 190 to 220 days before the birth of normal foals, and 210 to 240 days before the birth of most CHD foals. It may be that fetuses which are hypothyroid throughout gestation are more likely to develop flexural deformities at birth.

Various reports (Lowe and Kallfelz 1970; Rooney 1972; Shaver et al. 1979; Lokai and Ford 1981; McLaughlin and Doige 1981, 1982; Vivrette et al. 1984; McLaughlin et al. 1986; Allen et al. 1995) have implicated hypothyroidism as having a role in producing certain types of skeletal disease collectively referred to as developmental orthopedic disease (Pool 1993) in growing horses. Since the lesions of congenital hypothyroidism in foals have been described here, it should be easier to

recognize congenital hypothyroidism in foals in the future. An interesting prospective study would then be to monitor congenitally hypothyroid foals to see if they develop lesions of developmental orthopedic disease more often than other foals. If congenital hypothyroidism is found to have a role in the production of developmental orthopedic disease it will then be important to identify the causes of congenital hypothyroidism and possibly reduce the incidence of developmental orthopedic disease in the future .

6.0 A CASE-CONTROL STUDY OF NATURALLY OCCURRING CONGENITAL HYPOTHYROIDISM OF FOALS IN ALBERTA[1]

6.1 Introduction

Chapter 5.0 provided evidence that the syndrome of thyroid gland hyperplasia and musculoskeletal deformities (TH-MSD) of foals described in western Canada (McLaughlin and Doige 1981; McLaughlin et al. 1986; Kreplin and Allen 1991; Allen et al. 1994; Allen 1995) represents congenital hypothyroidism in foals. The cause of the congenital hypothyroidism remains unknown. However, a previous study (Allen et al. 1994) suggested that an investigation into different environmental exposures, particularly the feed, of the dams producing congenitally hypothyroid foals in western Canada was warranted. The purpose of this investigation was to identify risk factors for the development of congenital hypothyroidism in foals.

6.2 Materials and Methods

6.2.1 Subjects

A case-control study was conducted using privately owned foals born in Alberta in 1993. Members of the Alberta Standardbred Horse Association, the Alberta Division of the Canadian Thoroughbred Horse Society, the Western Canadian Association of

[1] Portions of this chapter have been published (Allen et al. 1996c).

Equine Practitioners, and the Alberta Veterinary Medical Association were informed of the study. They were requested to contact the investigator if a foal believed to be affected with the TH-MSD syndrome was born. Attempts were then made to identify a nearby farm expecting a similar number of foals in 1993, as a source of control foals.

A foal was classified as affected if it was examined by the investigator and found to have any 2 of the following 4 musculoskeletal anomalies: (1) any degree of mandibular prognathism; (2) flexural deformities of the legs; (3) rupture of 1 or both common digital extensor tendons; or (4) incomplete ossification of the carpal or tarsal bones. Ossification of the carpal or tarsal bones was assessed using radiographs and the skeletal ossification index presented in Table 3.1 (Adams and Poulos 1987, 1988; Adams 1990). The radiographs were evaluated independently of the investigator by a specialist and a resident in veterinary radiology. The radiologists were not given any information, except for the gestational age of the foal and the age of the foal at the time the radiographs were made. In addition, any thyroid gland that was available from any stillborn or dead TH-MSD foal was examined histologically for evidence of hyperplasia (Doige and McLaughlin 1981; McLaughlin and Doige 1981; Allen et al. 1994). A foal without the TH-MSD syndrome born to a mare that spent the majority of her gestation on an affected farm was referred to as an exposed foal. An affected farm was any farm where a mare producing an affected foal had spent most of her gestation.

A foal was classified as a control if it was thought to be normal by its owner or farm manager, found to be free of anomalies when examined by a local veterinary practitioner or the investigator, and born to a mare that had spent most of her gestation

on a farm that did not produce an affected foal. Farms that did not produce an affected foal were control farms.

6.2.2 Data collection

Information was collected to investigate the potential transmission of infectious agents, the likelihood of exposure to a toxic substance, and the possibility of a dietary deficiency. A questionnaire was used to assist with the collection of this information and was administered by the investigator during a personal interview with the owner or farm manager of each foal. Consultation with the local veterinary practitioner was sometimes required to complete the questionnaire.

For the purposes of this study, summer referred to the period from about June 1 to about September 30, 1992 and winter referred to the period from about October 1, 1992 until the time when mares foaled in 1993.

Whenever possible, samples of forage fed to pregnant mares were collected at time of the personal interview, and stored. Following the preliminary analysis of the data gathered for this study, these forage samples were analysed for nitrate levels using previously established methods (Helrich 1990).

6.2.3 Variables

The outcome or dependent variable was whether or not a foal used in the study was an affected (TH-MSD) or control foal. The independent variables investigated for

possible association with the TH-MSD syndrome have been summarized and appear as Appendix I.

The term greenfeed was used to refer to a cereal crop, almost always oats, harvested prior to maturity, i.e., "green", and baled for use as a livestock feed. Green oats, green oat hay, oat hay, green oat forage and oat straw are other terms that have been used to describe similar types of forage.

A variable representing the presence or absence of mineral supplementation of any kind was constructed from the variables concerned with feeding complete horse feed, feeding a protein-vitamin-mineral supplement, adding granular salt or mineral to grain, having free access to granular salt or mineral, and having free access to salt or mineral blocks.

6.2.4 Statistical analyses

The data set was checked for completeness and accuracy. Descriptive statistics were calculated for each of the continuous variables, and frequency tables were constructed for all categorical variables. Differences among farms and among mares producing affected and control foals were examined using Student's *t*-test, the chi-square test, and Fisher's exact test. Stratified analyses (Mantel-Haenszel) were performed to examine the association of various combinations of risk factors with the occurrence of disease (Mantel and Haenszel 1959). The analyses were performed using commercially available computer programs.

The association of all risk factors, except the presence of nitrate in forage samples, with the occurrence of disease was examined using all affected foals and a subset of control foals selected, using a random numbers table, from a list of all control foals. The probability of selection was proportional to the number of foals on each control farm, with all control farms being represented by at least 1 foal. The association between the presence of nitrate in forage samples and TH-MSD in foals was examined at the level of the farm and the individual animal. The later analysis included all foals examined on farms from which forage was collected.

6.3 Results

Fifty-four foals from 38 different farms were identified as affected foals. However, 15 foals from 12 farms could not be used in the study, as the owners or farm managers were either unable or unwilling to provide the information of interest. As a result, data from 186 foals comprised of 39 affected foals and 58 exposed foals from 26 affected farms, and 89 control foals from 23 control farms were available for analysis. The 39 affected foals included 2 aborted foals with lesions and farm histories consistent with the TH-MSD syndrome (Allen 1995). One hundred and twenty-four of the 186 foals received a detailed physical examination by the investigator, and 69 foals, including 36 of the 39 TH-MSD foals, were subjected to a radiographic examination of the carpal and tarsal regions.

The thyroid glands from 25 of the 39 affected foals were examined histologically. All of them were found to be hyperplastic and lacked normal amounts of

colloid. Only 10 of the 54 foals with TH-MSD were alive at the end of 1993. The remaining 44 foals were delivered dead, died, or were killed within a few days of birth.

A statistical comparison of affected and control farms found that the 2 groups of farms were similar in terms of size and animal signalment, except that standardbred mares and foals were moderately over represented on affected farms, relative to the control farms (Table 6.1). The mean number of years producing foals at the current location was considered equal ($P = 0.2349$). In both groups, there were 5 farms that were in their first foaling season on the present premises.

Of the 77 independent variables generated from the questionnaire, 10 were unconditionally associated with TH-MSD at a $P \leq 0.15$ level of significance. The other 67 independent variables were not pursued further.

Only gestation periods over 320 days were used in analyses and, as expected (Allen et al. 1993; 1994), length of gestation was found to be significantly ($P < 0.0001$) longer in the TH-MSD-affected foals compared to the sub-set of control foals (Table 6.2). Interestingly, the mean length of gestation (348.3 days) of exposed foals (n = 46) was significantly ($P < 0.0154$) longer than the mean gestation for all control foals (341.9 days, n = 63). Overall, the mean length of gestation (352.4 days) of all foals from affected farms (n = 75) was significantly ($P < 0.0001$) longer than control foals.

The association between each of the other 9 variables and TH-MSD are summarized in Table 6.3. Further analyses were stratified on the variables concerning the absence of any supplemental mineral during the winter and the feeding of greenfeed during the winter and are presented in Tables 6.4 and 6.5.

Table 6.1. Characteristics of affected and control farms with comparisons of the signalments of resident mares and foals

	Affected Farms	Control Farms	*P*-value
Total number of farms	26	23	
Total number of mares/foals	97	89	
Number of foaling mares per farm			
1	7 (26.9%)	7 (30.4%)	
2 to 5	14 (53.8%)	10 (43.5%)	
6 to 10	4 (15.4%)	4 (17.4%)	
11 to 15	1 (3.8%)	2 (8.7%)	
mean (*s*)	3.7 (2.8)	3.9 (3.7)	0.8822[a]
Age of foaling mares			
number (missing data)	91 (6)	73 (13)	
range	4 to 25	3 to 23	
mean (*s*)	10.0 (4.6)	10.6 (5.0)	0.3687[a]
Parity of foaling mares			
number (missing data)	90 (7)	82 (4)	
range	1 to 12	1 to 15	
mean (*s*)	3.8 (2.8)	4.3 (3.6)	0.3437[a]
Breed of foaling mares			
Arab	11 (11.3%)	6 (6.7%)	
other (includes mixed and Thoroughbred)	3 (3.1%)	13 (14.5%)	
Quarter horse	34 (35.1%)	36 (40.5%)	
Standardbred	49 (50.5%)	34 (38.2%)	0.0255[b]
Breed of foal			
Arab	10 (10.3%)	6 (6.7%)	
mixed	33 (34.0%)	35 (39.3%)	
other (includes Thoroughbred)	6 (6.2%)	14 (15.7%)	
Standardbred	48 (49.5%)	34 (38.2%)	0.0972[c]

Table 6.1. (continued)

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s = standard deviation
range = minimum and maximum values
[a]P-value associated with Student's t-test
[b]P-value associated with chi-square test after mixed, other and Thoroughbred were combined
[c]P-value associated with chi-square test after other and Thoroughbred were combined

Table 6.2. Length of gestation of congenitally hypothyroid foals and control foals

	Affected Foals	Control Foals	*P*-value
Number (missing data)	28 (11)	28 (11)	
Mean (*s*)	357.6 (11.7)	338.9 (10.8)	$< 0.0001^a$
Range	330 to 378	322 to 357	

s = standard deviation
[a]Two-sided *P*-value associated with Student's *t*-test
Range = the minimum and the maximum values greater than 320 days

Table 6.3. Categorical independent variables[a] unconditionally associated ($P \leq 0.15$) with the birth of congenitally hypothyroid foals

Variable	Affected	Control	Odds ratio	95% CI[b]	*P*-value[c]
greenfeed					
yes	10	1	13.1	1.6 to 108.3	0.0068
no	29	38			
left farm					
yes	19	7	4.3	1.5 to 12.2	0.0076
no	20	32			
irrigated pasture[d]					
yes	6	0	15.3	0.8 to 282.2	0.0254
no	33	39			
cattle on farm					
yes	19	9	3.2	1.2 to 8.4	0.0327
no	20	30			
no mineral					
yes	9	2	5.6	1.1 to 27.7	0.0472
no	30	37			
mineral block					
yes	22	31	0.3	0.1 to 0.9	0.0512
no	17	8			
other forage					
yes	7	15	0.4	0.1 to 1.0	0.0769
no	32	24			
creosote					
yes	11	19	0.4	0.2 to 1.1	0.1026
no	28	20			

Table 6.3. (continued)

Categorical independent variables[a] unconditionally associated ($P \leq 0.15$) with the birth of congenitally hypothyroid foals

Variable	Affected	Control	Odds ratio	95% CI[b]	*P*-value[c]
ivermectin[e]					
yes	14	22	0.4	0.2 to 1.1	0.1113
no	25	17			

[a]Variables as described in Appendix I
[b]Precision-based 95% confidence interval
[c]Two-sided *P*-value associated with Fisher's exact test
[d]To make calculations possible 0.5 was added to each value in the table
[e]Injectable ivermectin for cattle given orally

Table 6.4. Association of selected independent variables with the birth of congenitally hypothyroid foals after controlling for the effect of the absence of mineral supplementation in winter

Variable[a]	cOR[b]	sOR+[c]	sOR-[d]	chi-square[e]	*P*-value[f]	Summary ORmh[g]	95% CI[h]
greenfeed	13.1	0.3[i]	18	6.43[j]	< 0.025	na[k]	na[k]
left farm	4.3	0.4[i]	4.9	2.95	> 0.05	na[k]	na[k]
irrigated pasture	15.3	0.9[i]	16.2[i]	2.04[j]	> 0.1	na[k]	na[k]
other forage	0.4	0.1[i]	0.5	2.53	> 0.1	0.4	0.2 to 1.2
creosote	0.4	0.3[i]	0.6	0.12[j]	> 0.7	0.6	0.2 to 1.5
ivermectin[l]	0.4	0.1	0.6	0.76	> 0.3	0.5	0.2 to 1.3

[a]Variables as described in Appendix I
[b]Crude odds ratio used in Table 6.3
[c]Stratum specific odds ratio for dams that did not have regular access to supplemental minerals during the winter
[d]Stratum specific odds ratio for dams that had regular access to supplemental minerals during the winter
[e]Breslow-Day test for homogeneity of the odds ratio
[f]*P*-value associated with the Breslow-Day test for homogeneity with 1 degree of freedom
[g]Mantel-Haenszel summary odds ratio

Table 6.4. (continued)

[h]Test-based 95% confidence interval for the Mantel-Haenszel summary odds ratio
[i]Odds ratio calculated by adding 0.5 to all values in the stratum
[j]Breslow-Day test for homogeneity calculated after adding 0.5 to all values of those strata that contain a zero
[k]Use of a summary odds ratio (and 95% CI) is not appropriate when interaction is thought to be present
[l]Injectable ivermectin for cattle given orally

Table 6.5. Association of selected independent variables with the birth of congenitally hypothyroid foals after controlling for the effect of feeding greenfeed in winter

Variable[a]	cOR[b]	sOR+[c]	sOR-[d]	chi-square[e]	*P*-value[f]	Summary ORmh[g]	95% CI[h]
left farm	4.3	0.1[i]	7.6	12.14	< 0.005	na[j]	na[j]
irrigated pasture	15.3	0.1[i]	21.3[i]	8.53[k]	< 0.005	na[j]	na[j]
cattle on farm	3.2	1.1[i]	2.3	0.52	> 0.4	2.1	0.7 to 6.1
no mineral	5.6	0.1[i]	8.1	6.27[k]	< 0.025	na[j]	na[j]

[a]Variables as described in Appendix I
[b]Crude odds ratio used in Table 6.3
[c]Stratum specific odds ratio for dams fed greenfeed during the winter
[d]Stratum specific odds ratio for dams not fed greenfeed during the winter
[e]Breslow-Day test for homogeneity of the odds ratio
[f]*P*-value associated with the Breslow-Day test for homogeneity with 1 degree of freedom
[g]Mantel-Haenszel summary odds ratio
[h]Test-based 95% confidence interval for the Mantel-Haenszel summary odds ratio
[i]Odds ratio calculated by adding 0.5 to all values in the stratum

Table 6.5. (continued)

[j]Use of a summary odds ratio (and 95% CI) is not appropriate when interaction is thought to be present
[k]Breslow-Day test for homogeneity calculated after adding 0.5 to all values of those strata that contain a zero

The association between TH-MSD and variables reflecting the feeding of forage other than hay, greenfeed, or silage; the use of creosote on fences and buildings; and the use of injectable ivermectin for cattle administered orally to horses, lacked statistical significance and did not reveal interaction or confounding after controlling for the effects of no mineral supplementation (Table 6.4). The same was true for the presence of cattle on the farms used in this study, after controlling for the use of greenfeed in the winter (Table 6.5).

The association between mares grazing irrigated pasture and TH-MSD could not be interpreted and further evaluation could not be undertaken as the data were too sparse. Specifically, only 6 of the 78 dams grazed irrigated pasture and all 6 produced TH-MSD syndrome foals. Only 1 of these 6 dams also failed to receive mineral supplementation and none of the 6 dams were exposed to greenfeed.

An examination of "left farm" in Tables 6.4 and 6.5 reveals that its association with TH-MSD varies with the presence or absence of supplemental mineral and with the feeding of greenfeed.

Samples of 20 different forages from 14 of the 26 affected farms and of 10 different forages from 7 of the 23 control farms were collected. Nitrate was present more often (8 of 14 farms) and at higher concentrations in those samples collected from affected farms compared to those from control farms (1 of 7). The odds of at least 1 case of the TH-MSD syndrome occurring on farms feeding forage with at least a trace of nitrate was 8.0 times greater ($P = 0.0873$) than the odds of disease occurring on farms that fed forage free of nitrate. On an individual animal basis, the odds of a mare

producing a TH-MSD foal when exposed to forage containing at least a trace of nitrate was 5.9 times greater ($P = 0.0007$) than for mares exposed to nitrate-free forage.

6.4 Discussion

The case-control method of investigation has been widely accepted as the research strategy of choice when initiating an exploratory study of disease etiology (Schlesselman and Stolley 1982; Rothman 1986; Martin et al. 1987; Greenberg and Ibrahim 1991). Thomas et al. (1985) have recommended that all associations under study should be reported so that they are open to scrutiny by the reader. All variables examined in this study can be found, in abridged form, in Appendix I. Those variables deemed appropriate for additional study were presented in Table 6.3.

The major concern associated with case-control studies is the potential for systematic errors or bias (Schlesselman and Stolley 1982; Rothman 1986; Martin et al. 1987; Greenberg and Ibrahim 1991). The most likely source of potential bias in this study would have been differential misclassification of either disease or exposure status. Given the obvious nature of the specific and uncommon combination of lesions required to classify a foal as affected, misclassification of disease status seemed unlikely. In addition, the histologic appearance of the thyroid gland was used to support the diagnosis of the TH-MSD syndrome in foals with lesions consistent with the case definition.

The potential for misclassification of exposure status was a greater concern. However, if misclassification had occurred with regard to the feeding of greenfeed or

the failure to supplement mares with mineral in winter, it would have acted to decrease the strength of association of these variables with the disease and the true odds ratios would have been even greater than those reported here. The author believed that most horse producers would have viewed the feeding of greenfeed as a nontraditional and questionable practice. The author also believed that most horse producers would have felt that supplementing the diet of pregnant mares with minerals was beneficial and should have been done. For these reasons, we felt that participants in this study would, if anything, have been inclined to under-report the feeding of greenfeed and the failure to supplement mineral. In this situation, the study would have produced an underestimate of the true exposure-disease association.

The feeding of greenfeed in winter had a strong (OR = 13.1) and highly significant ($P = 0.0068$) measure of association with TH-MSD. The potential for greenfeed to accumulate high levels of nitrate (NO_3^-) and nitrite (NO_2^-) has been well recognized (Newsom et al. 1937; Thorp 1938; Bradley et al. 1939a, 1939b, 1940; Riggs 1945; Crawford et al. 1966; Dollahite and Holt 1969; Neilson 1974; Smith and Suleiman 1991). In fact, prior to identifying nitrate as the causative agent of methemoglobinemia (nitrate poisoning) of cattle, the condition was referred to as oat hay poisoning (Newsom et al. 1937; Thorp 1938; Bradley et al. 1939a, 1939b, 1940). Smith and Suleiman (1991) have reported that Alberta producers often provide high-nitrate feeds, including oat greenfeed, to livestock. Reviews of nitrate and nitrite toxicity in animals have been published previously (Case 1957; Wright and Davison 1964; Ridder and Oehme 1974; Johnson et al. 1983; Osweiler et al. 1985; Bruning-Fann

and Kaneene 1993). Pertinent to this discussion is the association between nitrate exposure and alterations in iodine metabolism, thyroid activity or thyroid gland morphology reported in a variety of animals including fish (Lahti et al. 1985), rats (Wyngaarden et al. 1952, 1953; Bloomfield 1961; Welsch et al. 1961, 1962; Yadav et al. 1962; Lee et al. 1970; Horning et al. 1986; Jahreis 1989), growing pigs (Dvorak and Neumannova 1986; Jahreis et al. 1986a, 1986b, 1987), goats (Prasad 1983), lambs and sheep (Bloomfield et al. 1961, 1962; Cline et al. 1963; Arora et al. 1966, 1968; Carver and Pfander 1973; Korber et al. 1983; Georgiev et al. 1987), and cattle (Korber et al. 1983). Further, there is correlational evidence suggesting that a high level of nitrate present in drinking water is associated with an elevated rate of goitre in people in specific regions of Germany (Sauerbrey et al. 1989) and Nigeria (Ubom 1991). It has also been shown that nitrate is able to cross the placenta of rats (Gruener et al. 1973; Hirneth and Classen 1984), guinea pigs (Sinha and Sleight 1971), pigs (Garner et al. 1958) and cattle (Malestein et al. 1980; Osweiler et al. 1985; Slanina et al. 1990; Johnson et al. 1992). Interestingly, ingestion of nitrate by pregnant cows has been implicated as a cause of congenital arthrogryposis (Johnson et al. 1983), as well as prolonged gestation, depressed thyroxine levels, and enlarged thyroid glands (Pethes et al. 1983) in their calves.

If nitrate present in the diet of pregnant mares is able to cross the placenta and interfere with fetal thyroid function, then it will be important to consider all sources of environmental nitrate in order to fully understand and prevent disease. There can be considerable variation in the nitrate levels found in plants due to the plant species, stage

of maturity, nitrogen content of the soil and water, and other growing conditions. Many of the plants commonly made available to horses in western Canada can accumulate high levels of nitrate; these include alfalfa, timothy, ryegrass, sweet clover, and a wide variety of weeds. Many cereal crops, such as oats, wheat, barley, rye, corn, and flax, are also able to concentrate nitrate. Different parts of the plant contain different levels of nitrate, and very little is present in the seed or grain. As nitrate is water soluble, water can also be an important source of nitrate for livestock, particularly near areas of heavy fertilization, feedlots, dairies, landfills, and some types of industry. The potential for problems is likely to be increased following periods of high surface runoff created by the melting of snow in spring, heavy rain fall, or irrigation. It is worth emphasizing that nitrate in feed, water, and other potential sources is additive in its effect, and all sources will have to be considered when investigating a suspected nitrate problem (Wright and Davison 1964; Dollahite and Holt 1969; National Research Council Committee on Nitrate Accumulation 1972; Ridder and Oehme 1974; O'Hara and Fraser 1975; Johnson et al. 1983; Osweiler et al. 1985; Johnson et al. 1992; Bruning-Fann and Kaneene 1993).

An absence of supplemental salt or mineral during the winter had a moderately strong measure of association (OR = 5.6) with TH-MSD, and this association was probably underestimated, since no attempt was made to estimate the quantity or the quality of the mineral consumed on a farm or individual animal basis. Despite this, the relationship was statistically significant ($P = 0.0472$) and a biologically plausible association among a deficiency of minerals, thyroid function, and the TH-MSD syndrome has been discussed previously (Allen et al. 1994). Iodine is essential for

normal thyroid function, and failure to supplement pregnant mares with mineral may have been associated with an iodine deficiency since western Canadian soils and the plants grown on these soils are believed to be very low in iodine.

Information concerning a mare's movement off and onto her "home farm" was included as 1 of several variables to evaluate the possibility of an infectious agent being the cause of the TH-MSD syndrome. However, the failure of other related variables to be associated with TH-MSD invites a different interpretation of the results that were obtained. An examination of "left farm" in Tables 6.5 and 6.6 demonstrates that the association between movement off and onto the "home farm" and TH-MSD varies with the presence or absence of supplemental mineral and the feeding of greenfeed. This effect, referred to as interaction, supports the conclusion that feeding greenfeed and failing to provide supplemental minerals during the winter were risk factors for disease. It would appear that dams which left a high risk environment, i.e., one that failed to provide supplemental mineral or that fed greenfeed, had a reduced risk of producing a TH-MSD foal; while dams which left a low risk environment had an increased risk of producing a TH-MSD foal.

Unfortunately, the combined effects of the lack of supplemental mineral and the feeding of greenfeed on the occurrence of the TH-MSD syndrome could not be pursued with this data set as none of the farms included in this study fed greenfeed and failed to provide supplemental mineral during the winter. There is evidence in rats (Lee et al. 1970) and in pigs (Jahreis et al. 1986a) that increased levels of iodine in the diet can counteract, to some degree, the effects of nitrate on thyroid activity.

This study has identified that the lack of mineral supplementation and the presence of greenfeed in the diet of pregnant mares significantly increased the risk of producing a TH-MSD syndrome foal. It has been argued that the ingestion of nitrate and a deficiency of iodine are 2 underlying factors which produce disease by interfering with fetal thyroid function.

While this study has been successful in generating new hypotheses about the cause or causes of the TH-MSD syndrome, it will be important to test these hypotheses through additional epidemiologic investigations (Thomas et al. 1985) and controlled experiments.

7.0 GENERAL DISCUSSION AND CONCLUSIONS

7.1 Introduction

This thesis represents a series of investigations where the results of each study not only made a contribution within a given discipline, but also provided information on which a successive study could be built. A summary of these results and a discussion of their role in the broader understanding of congenital hypothyroidism of foals follows.

7.2 Thyroid Gland Lesions, Hypothyroidism, and Thyroid Function Testing in Horses

In the introduction to this thesis it was noted that, in the veterinary literature, there was an apparent lack of understanding of the relationship between morphological lesions and functional lesions of the thyroid gland; and a need for consistency in the terminology used to describe the nature of thyroid gland lesions of horses. For example, the traditional use of the word goitre was to denote any abnormality, structural or functional, of the thyroid gland. More recently, goitre has come to be defined as an enlargement of the thyroid gland, regardless of the nature of that enlargement.

There was no attempt in this thesis to address these issues directly. However, the congenitally hypothyroid foals in these studies served as examples that thyroid

glands that appear normal grossly may not function appropriately and, conversely, that hypothyroid foals are not necessarily goitrous.

Portions of this thesis have also discussed the problems veterinarians currently face in the diagnosis of hypothyroidism. The author believes that efficiency in the diagnosis or exclusion of hypothyroidism in horses may be gained if thyroid function testing is limited to animals suspected of being hypothyroid. For this reason, the literature concerning hypothyroidism in horses was reviewed in an attempt to record the clinical signs which should and should not raise suspicion of the presence of the disease.

Hypothyroidism was surgically created in horses and ponies by Lowe and others at Cornell University (Lowe and Kallfelz 1970; Lowe et al. 1974, 1975, 1987). They found that, compared to control horses kept under similar conditions, hypothyroid horses were docile and lethargic; sensitive to cold; failed to grow and mature; developed a dull, coarse hair coat that was late to shed and became sparse; exhibited thickened, wrinkled skin, particularly over the face; developed edema of the rear limbs; and had lower rectal temperatures, lower hematocrits, and higher blood cholesterol levels. There are also two case reports which provide convincing evidence of naturally occurring hypothyroidism in horses (Stanley and Hillidge 1982; Hillyer and Taylor 1992). In these reports, the clinical signs are limited to or primarily concerned with the hair coat and skin.

There is little evidence to support claims that hypothyroidism is the cause of agalactia, anhidrosis, "cresty" or thickened necks, infertility, laminitis, obesity, or rhabdomyolysis (Britton 1959; Smythe 1963; Correa and Calderin 1966; Waldron-

Mease 1979; Hillenbrand 1991; Smith 1994). These issues have been addressed in greater depth in Chapter 1.

This thesis has also described the prolonged gestation and difficult parturition associated with congenital hypothyroidism in foals, and has clearly documented the congenital lesions present which confirm the diagnosis.

This having been stated, the need for a readily accessible, reasonably priced, and reliable test of thyroid function in horses remains as great today as it was 30 years ago when this need was recognized by Irvine (1966) in a presentation to the American Association of Equine Practitioners. The difficulties in interpreting resting triiodothyronine (T_3) and thyroxine (T_4) levels of horses have also been summarized (Chapter 2) and argue against using these values as the sole means of diagnosis.

Dybdal (1996) has commented that many papers exist which describe the response of normal horses to thyroid-stimulating hormone (TSH) or thyrotropin-releasing hormone (TRH) administration and propose that any horse that does not respond in a similar manner is hypothyroid. His concern is that no reports exist which have demonstrated that horses, known to be hypothyroid, fail to meet these criteria when tested. However, this observation appears to have overlooked the work of Lowe and his colleagues (Lowe and Kallfelz 1970; Lowe et al. 1974; 1987) and, in the presence of this work, the case reports of Stanley and Hillidge (1982), and Hillyer and Taylor (1992).

Information regarding thyroid function testing in neonatal foals is even more limited (Shaftoe et al. 1988) than that available for adult horses. Therefore, the studies

reported here (Chapter 2) have contributed to this area of equine neonatology and are in agreement with those of Shaftoe et al. (1988). Both works demonstrated that the TSH response test can be used effectively to confirm a diagnosis of hypothyroidism in neonatal foals. Foals in these studies that were suspected of being hypothyroid, i.e., foals with thyroid gland hyperplasia and musculoskeletal deformities (TH-MSD), had similar lesions and responses to TSH administration as surgically created hypothyroid foals.

The concerns that TSH is expensive and not readily available and that few laboratories are equipped for and experienced with measuring thyroid hormones in equine samples (Sojka 1993; Messer 1995b; Sojka and Levy 1995; Dybdal 1996) are shared by this author. Fortunately, the Endocrine Laboratory at the Western College of Veterinary Medicine (WCVM) has the instrumentation to assay thyroid hormones and the assay was validated for use in these investigations. The finding that the assay could be performed on blood samples that were held at room temperature for a least 24 hours allowed for the collection of samples from horses located outside the WCVM (Chapter 2; Chapter 4).

The best hope for a practical and reliable test of thyroid function in the horse is for the development of a species-specific assay for TSH (Beech 1987), something that has revolutionized the way disorders of thyroid function are diagnosed in people (Larsen and Ingbar 1992; Ferguson 1994; Feldman and Nelson 1996), and that has been anxiously anticipated by canine endocrinologists for some time (Ferguson 1984; Ferguson 1994; Feldman and Nelson 1996).

7.3 Congenital Hypothyroidism in Western Canada, the United States of America, and Elsewhere

With this thesis, the body of knowledge documenting naturally occurring cases of congenitally hypothyroid foals in western Canada is substantially increased. Congenital hypothyroidism was the most common underlying problem of foals admitted to the Veterinary Teaching Hospital of the WCVM during the period of this thesis research. It appears appropriate therefore to suggest that congenital hypothyroidism has been an important cause of foal mortality, reproductive loss, and decreased productivity in the western Canadian horse industry (Chapter 3). There is also a published report strongly suggesting the presence of congenitally hypothyroid foals in the northern United States as early as 1975 (Myers and Gordon 1975), and, very recently, a definitive description of over 20 affected foals in the western United States (Hines et al. 1996). These reports, combined with numerous, reliable, personal communications received by the author, indicate that hypothyroid foals are born periodically throughout the northwestern United States (Michigan, Wisconsin, Minnesota, North Dakota, Montana, Idaho, Washington, Oregon, and northern California) and, at least occasionally, in western Ontario, Australia, and possibly Finland (Ruohoniemi 1993).

If the theories suggesting that the presence of nitrate and the deficiency of iodine in the diet of pregnant mares are causes of congenital hypothyroidism in western Canadian foals are accurate (Chapter 6), then the concern exists that the same dietary factors have the potential to cause problems in other livestock (cattle, sheep, pigs and poultry) raised in the same areas as affected foals.

7.4 The Importance of Normal Thyroid Function in the Perinatal Foal

The study of thyroid hormone levels present in equine fetuses at different stages of gestation (Chapter 4) clearly demonstrated that there is significant thyroid activity during the last third of gestation, but that late term fetuses do not have T_3 levels as high as those of neonates. Therefore, it was concluded that there must be a stimulus very near to parturition, that results in an increase of fetal T_3 concentrations. The nature of this stimulus and the mechanism behind the rapid increase remains unknown, but is potentially of interest to equine neonatologists since there is also a rapid rise in fetal adrenocorticotropic hormone and cortisol levels near parturition (Silver et al. 1991; Cudd et al. 1995). The possible relationship between the rise in cortisol and T_3 in the periparturient fetus, and the relationship of these changes to fetal maturation and the initiation of parturition are intriguing questions since, in most other species studied, increased activity of the hypothalamic-pituitary-adrenal axis mediates these processes (Cudd et al. 1995).

It has also been noted that neonatal foals have thyroid hormone levels higher than those of any other animal at any physiological state (Irvine and Evans 1975). This has led to speculation that thyroid hormone plays an important role in the some aspect of equine neonatal function and development which is critical for survival. The high mortality rate of the congenitally hypothyroid foals in these studies supports this theory. It has also been shown that premature foals exhibit hypoadrenocorticism (Silver et al. 1984). Given that the administration of adrenocorticotropic hormone to fetal foals

produces a rise in plasma T_3 levels (Silver et al. 1991), it has been suggested that the administration of thyroid hormones may be useful in the treatment of premature foals.

These studies, those of Lowe et al. (Lowe and Kallfelz 1970; Lowe et al. 1974; 1987), and unpublished observations of this author have also shown that normal thyroid function is critical for normal skeletal development in the horse and that hypothyroid foals will develop several forms of skeletal disease referred to as developmental orthopedic disease (Pool 1993). The role of altered thyroid function in the pathogenesis of other types of orthopedic disease remains to be determined.

7.5 Testable Hypotheses

This thesis and, more specifically, this discussion have touched on several areas where the information generated from this work may contribute to the understanding, and also pose a number of interesting questions, of various aspects of equine neonatology. However, the only stated objective of this thesis regarding future studies (Chapter 1) was to provide testable hypotheses about the cause of naturally occurring congenital hypothyroidism in foals (Chapter 6).

Clearly, the next set of investigations should test the hypotheses that pregnant mares which have ingested nitrate or have received insufficient iodine during gestation produce congenitally hypothyroid foals. The minimum level of dietary iodine required to prevent disease has not been investigated. Although the National Research Council (1989) has guidelines for the consumption of iodine by pregnant mares, these recommendations are based on rather old, and, in the opinion of this author, imprecise

observations (Rodenwold and Simms 1934). More closely controlled studies on the iodine requirements of pregnant mares are clearly indicated.

7.6 Concluding Remarks

The purpose of these investigations was not to simply amass data on the problems of skeletal anomalies, dysmaturity or congenital hypothyroidism in foals, but rather to try to understand the pathogenesis of these problems, and if possible, to determine their cause. It was believed that acquiring knowledge about the cause and pathogenesis would allow for the prevention of what was, at first, known descriptively as TH-MSD, and what was, in the end, determined to be congenital hypothyroidism of foals. It was also hoped that an understanding of the cause and pathogenesis of the TH-MSD syndrome would provide the means to treat affected foals more effectively. Now that the pathogenesis and risk factors for disease have been identified more logical approaches to therapy aimed at negating the effects of the etiologic agent, correcting for apparent deficiencies, or both, can be adopted.

8.0 REFERENCES

Abbott AC, Prendergast J. Histological variations in animal thyroids in western Canada. Journal of the Canadian Medical Association 1934; 31: 465-473.

Adams R. Noninfectious orthopedic problems. In: Koterba AM, Drummond WH, Hosch PC, eds. Equine Clinical Neonatology. Philadelphia: Lea & Febiger, 1990: 333-366.

Adams R, Poulos P. Radiographic evaluation of the carpal and tarsal regions of neonatal foals. Proceedings of the 33rd Annual Convention of the American Association of Equine Practitioners 1987: 677-682.

Adams R, Poulos P. A skeletal ossification index for neonatal foals. Veterinary Radiology 1988; 29: 217-222.

Allen AL. Hyperplasia of the thyroid gland and musculoskeletal deformities in two equine abortuses. Canadian Veterinary Journal 1995; 36: 234-236.

Allen AL, Doige CE, Fretz PB, Townsend HGG, Card CE. Congenital hypothyroidism, dysmaturity and musculoskeletal lesions in western Canadian foals. Proceedings of the 39th Annual Convention of the American Association of Equine Practitioners 1993: 207-208.

Allen AL, Doige CE, Fretz PB, Townsend HGG. Hyperplasia of the thyroid gland and concurrent musculoskeletal deformities in western Canadian foals: Reexamination of a previously described syndrome. Canadian Veterinary Journal 1994; 35: 31-38.

Allen AL, Doige CE, Haas SD, Card CE, Fretz PB. Concentrations of triiodothyronine and thyroxine in equine fetal serum during gestation. In: Sharp DA, Brazer FW, eds. Equine Reproduction. Madison, Wisconsin,: Society for the Study of Reproduction, 1995: 49-52. (Biology of Reproduction Monograph Series 1, volume VI).

Allen AL, Fretz PB, Card CE, Doige CE. The effect of partial thyroidectomy on the development of the equine foetus. Equine Veterinary Journal 1996a; (accepted for publication).

Allen AL, Scott WM, Cook SJ, Fretz PB, Doige CE. Effects of delayed serum separation and long-term storage on the measurement of thyroid hormones in blood samples from horses. Veterinary Clinical Pathology 1996b; (in press).

Allen AL, Townsend HGG, Doige CE, Fretz PB. A case-control study of the congenital hypothyroidism and dysmaturity syndrome of foals. Canadian Veterinary Journal 1996c; 37: 349-351, 354-358.

Arnold U, Opitz M, Grosser I, Bader R, Eigenmann JE. Goitrous hypothyroidism and dwarfism in a kitten. Journal of the American Animal Hospital Association 1984; 20: 753-758.

Arora SP, Hatfield EE, Romack FE, Garrigus US. Effect of dietary nitrate upon I^{131} uptake by thyroids in lambs. Journal of Animal Science 1966; 25: 1253.

Arora SP, Hatfield EE, Garrigus US, Romack FE, Motyka H. Effect of adaptation to dietary nitrate on thyroxine secretion rate and growth in lambs. Journal of Animal Science 1968; 27: 1445-1448.

Baker HJ, Lindsey JR. Equine goiter due to excess iodide. Journal of the American Veterinary Medical Association 1968; 153: 1618-1630.

Baker JR, Wyn-Jones G, Eley JL. Case of equine goitre. The Veterinary Record 1983; 112: 407-408.

Bath GF, Wentzel D, Van Tonder EM. Cretinism in Angora goats. Journal of the South African Veterinary Association 1979; 50: 237-239.

Becks H, Scow RO, Simpson ME, Asling CW, Li CH, Evans HM. Response by the rat thyro-parathyroidectomized at birth to growth hormone and to thyroxine given separately or in combination. The Anatomical Record 1950; 107: 299-318.

Becks H, Simpson ME, Scow RO, Asling CW, Evans HM. Skeletal changes in rats thyroidectomized on the day of birth and the effects of growth hormone in such animals. The Anatomical Record 1948; 100: 561-576

Beech J. Evaluation of thyroid, adrenal, and pituitary function. Veterinary Clinics of North America: Equine Practice 1987; 3: 649-660.

Beech J, Garcia M. Hormonal response to thyrotropin-releasing hormone in healthy horses and in horses with pituitary adenoma. American Journal of Veterinary Research 1985; 46: 1941-1943.

Beech J, Garcia M. Diseases of the thyroid gland. In: Colahan PT, Mayhew IG, Merritt AM, Moore JN, eds. Equine Medicine and Surgery. 4th ed. vol 2. Goleta, California: American Veterinary Publications, 1991: 1737-1751.

Behrman RE, ed. Nelson Textbook of Pediatrics. 14th ed. Toronto: WB Saunders, 1992: 1416-1430.

Blackmore DJ, Greenwood RES, Johnson C. Observations on thyroid hormones in the blood of thoroughbreds. Research in Veterinary Science 1978; 25: 294-297.

Bloomfield RA, Welsch CW, Garner GB, Muhrer ME. Thyroidal I^{131} metabolism in nitrate fed sheep. Journal of Animal Science 1962; 21: 988.

Bloomfield RA, Welsch CW, Garner GB, Muhrer ME. Effect of dietary nitrate on thyroid function. Science 1961; 134: 1690.

Boosinger TR, Brendemuehl JP, Bransby DL, Wright JC, Kemppainen RJ, Kee DD. Prolonged gestation, decreased triiodothyronine concentration, and thyroid gland histomorphologic features in newborn foals of mares grazing Acremonium coenophialum-infested fescue. American Journal of Veterinary Research 1995; 56: 66-69.

Bradley WB, Beath OA, Eppson HF. Oat hay poisoning. Science 1939a; 89: 365.

Bradley WB, Eppson HF, Beath OA. Nitrate as the cause of oat hay poisoning. Journal of the American Veterinary Medical Association 1939b; 94: 541-542.

Bradley WB, Eppson HF, Beath OA. Methylene blue as an antidote for poisoning by oat hay and other plants containing nitrates. Journal of the American Veterinary Medical Association 1940; 96: 41-42.

Britton JW. Spontaneous equine laminitis. California Veterinarian 1959; 13: 17-18.

Bruning-Fann CS, Kaneene JB. The effects of nitrate, nitrite, and N-nitroso compounds on animal health. Veterinary and Human Toxicology 1993; 35: 237-253.

Bustad LK, Fuller JM. Thyroid function in domestic animals. Laboratory Animal Care 1970; 20: 561-581.

Carver LA, Pfander WH. Urea utilization by sheep in the presence of potassium nitrate. Journal of Animal Science 1973; 36: 581-587.

Case AA. Some aspects of nitrate intoxication in livestock. Journal of the American Veterinary Medical Association 1957; 130: 323-329.

Chard T. An introduction to radioimmunoassay and related techniques, 3rd ed. New York: Elsevier, 1987: 161-174, 194-214. (Burdon RH, van Knippenberg PH, eds; Laboratory techniques in biochemistry and molecular biology, volume 6, part 2).

Chastain CB. Canine pseudohypothyroidism and covert hypothyroidism. Problems in Veterinary Medicine 1990; 2: 693-716.

Chastain CB, McNeel SV, Graham CL, Pezzanite SC. Congenital hypothyroidism in a dog due to an iodide organification defect. American Journal of Veterinary Research 1983; 44: 1257-1265.

Chastain CB, Panciera DL. Hypothyroid diseases. In: Ettinger SJ, Feldman EC, eds. Textbook of Veterinary Internal Medicine. 4th ed. vol 2. Philadelphia: WB Saunders, 1995: 1487-1501.

Chapman RE, Hopkins PS, Thorburn GD. The effects of fetal thyroidectomy and thyroxine administration on the development of the skin and wool follicles of sheep fetuses. Journal of Anatomy 1974; 117: 419-432.

Chen CL, Li WI. Effect of thyrotropin releasing hormone (TRH) on serum levels of thyroid hormones in Thoroughbred mares. Equine Veterinary Science 1985; 6: 58-61.

Chen CL, Riley AM. Serum thyroxine and triiodothyronine concentrations in neonatal foals and mature horses. American Journal of Veterinary Research 1981; 42: 1415-1417.

Chen CL, McNulty ME, McNulty PK, Asbury AC. Serum levels of thyroxine and triiodothyronine in mature horses following oral administration of synthetic thyroxine (Synthroid^{R}). Journal of Equine Veterinary Science 1984; 4: 5-6.

Chew FS. Radiologic manifestations in the musculoskeletal system of miscellaneous endocrine disorders. Radiology Clinics of North America 1991; 29: 135-147.

Cline TR, Hatfield EE, Garrigus US. Effects of potassium nitrate, alpha-tocopherol, thyroid treatments, and vitamin A on weight gain and liver storage of vitamin A in fattening lambs. Journal of Animal Science 1963; 22: 911-913.

Conway DA, Cosgrove JS. Equine goitre. Irish Veterinary Journal 1980; 34: 29-31.

Correa JE, Calderin GG. Anhidrosis, dry-coat syndrome in the Thoroughbred. Journal of the American Veterinary Medical Association 1966; 149: 1556-1560.

Crawford, Kennedy WK, Davison KL. Factors influencing the toxicity of forages that contain nitrate when fed to cattle. Cornell Veterinarian 1966; 56: 3-17.

Cudd TA, LeBlanc M, Silver M, Norman W, Madison J, Kellor-Wood M, Wood CE. Ontogeny and ultradian rhythms of adrenocorticotropin and cortisol in the late-gestation fetal horse. Journal of Endocrinology 1995; 144: 271-283.

Dalefield RR, Palmer DN. The frequent occurrence of thyroid tumors in aged horses. Journal of Comparative Pathology 1994; 110: 57-64.

DeLellis RA. The endocrine system. In: Cotran RS, Kumar V, Robbins SL, eds. Robbins Pathologic Basis of Disease. 4th ed. Toronto: WB Saunders, 1989: 1205-1276.

Diamond FB, Root AW. Thyroid function in the preterm infant. In: Bercu BB, Shulman DI, eds. Advances in perinatal thyroidology. New York: Plenum Press, 1991: 227-241.

Dimock WW, Westerfield C, Doll ER. The equine thyroid in health and disease. Journal of the American Veterinary Medical Association 1944; 104: 313-317.

Doige CE, McLaughlin BG. Hyperplastic goitre in newborn foals in western Canada. Canadian Veterinary Journal 1981; 22: 42-45.

Dollahite JW, Holt EC. Nitrate poisoning. Southwestern Veterinarian 1969; 23: 23-28.

Drew B, Barber WP, Williams DG. The effects of excess dietary iodine on pregnant mares and foals. The Veterinary Record 1975; 97: 93-95.

Driscoll J, Hintz HF, Schryver HF. Goiter in foals caused by excessive iodine. Journal of the American Veterinary Medical Association 1978; 173: 858-859.

Duckett WM, Manning JP, Weston PG. Thyroid hormone periodicity in healthy adult geldings. Equine Veterinary Journal 1989; 21: 123-125.

Dudan DC, Ferguson DC, Little TV, Livesay-Wilkins P, Nathanielsz PW, Hillman RB. Circulating serum thyroxine (T4), triiodothyronine (T3) and reverse T3 (rT3) in neonatal term and preterm foals. Proceedings of the 5th American College of Veterinary Internal Medicine Forum 1987: 881.

Durham AE. Congenital goitre in two colt foals born to mares fed excess iodine during pregnancy. Equine Veterinary Education 1995; 7: 239-241.

Dvorak M, Neumannova M. Rust odstavenych selat a hladina jodtyroninu v krevnim seru pri pusobeni tyreoaktivnich latek. Veterinari Medicina (Praha) 1986; 31: 265-276.

Dybdal NO. Thyroid gland. In: Smith BP. Large Animal Internal Medicine. 2nd ed. St. Louis: Mosby-Year Book, 1996: 1451-1453.

Feldman EC and Nelson RW. Canine and Feline Endocrinology and Reproduction, 2nd ed. Toronto: WB Saunders, 1996: 68-117.

Ferlazzo A, Longo A, Panzera M, Zannelli C. Serum triiodothyronine and thyroxine in mares before and after foaling. In: Blackmore DJ, ed. Animal Clinical Biochemistry-The Future. Cambridge: Cambridge University Press, 1988: 269-273.

Ferguson DC. Thyroid function tests in the dog. Veterinary Clinics of North America: Small Animal Practice 1984; 14: 783-808.

Ferguson DC. The effect of nonthyroidal factors on thyroid function tests in dogs. Compendium on Continuing Education for the Practicing Veterinarian 1988; 10:1365-1370, 1372-1373, 1376-1377.

Ferguson DC. Update on the diagnosis of canine hypothyroidism. Veterinary Clinics of North America: Small Animal Practice 1994; 24: 515-539.

Ferguson KA, Schinckel PG, Carter HB, Clarke WH. The influence of the thyroid on wool follicle development in the lamb. Australian Journal of Biological Sciences 1956; 9: 575-585.

Fisher DA, Dussault JH, Lam RW. Serum and thyroid gland triiodothyronine in the human fetus. Jounal of Clinical Endocrinology and Metabolism 1973; 36: 397-400.

Fisher DA, Dussault JH, Sack J, Chopra IJ. Ontogenesis of hypothalamic-pituitary-thyroid function and metabolism in man, sheep, and rat. Recent Progress in Hormone Research 1977; 33: 59-116.

Flisińska-Bojanowska A, Komosa M, Gill J. Influence of pregnancy on diurnal and seasonal changes in cortisol, T_3 and T_4 levels in the mare blood serum. Comparative Biochemistry and Physiology 1991; 98A: 23-30.

Fretz PB. Angular limb deformities in foals. Veterinary Clinics of North America: Large Animal Practice 1980; 2: 125-150.

Fretz PB. Congenital hypothyroidism-dysmaturity syndrome in western Canadian foals: clinical manifestations, management, treatment, prognosis, and follow up. Proceedings of the 40th Annual Convention of the American Association of Equine Practitioners 1994: 63.

Fretz PB, Donnecker JM. Surgical correction of angular limb deformities in foals: a retrospective study. Journal of the American Veterinary Medical Association 1983; 183: 529-532.

Fretz PB, McIlwraith. Wedge osteotomy as a treatment for angular deformity of the fetlock in horses. Journal of the American Veterinary Medical Association 1983; 182: 245-250.

Fretz PB, Turner AS, Pharr J. Retrospective comparison of two surgical techniques for correction of angular limb deformities in foals. Journal of the American Veterinary Medical Association 1978; 172: 281-286.

Fretz PB, Cymbaluk NF, Pharr JW. Quantitative analysis of long-bone growth in the horse. American Journal of Veterinary Research 1984; 45: 1602-1609.

Furr MO, Murray MJ, Ferguson DC. The effects of stress on gastric ulceration, T3, T4, reverse T3 and cortisol in neonatal foals. Equine Veterinary Journal 1992; 24: 37-40.

Garcia MC, Beech J. Endocrinologic, hematologic, and heart rate changes in swimming horses. American Journal of Veterinary Research 1986; 47: 2004-2006.

Garner GB, O'Dell BL, Radar P, Muhrer ME. Further studies on the effects of nitrate upon reproduction and vitamin A storage with rats and swine. Journal of Animal Science 1958; 17: 1213.

Georgiev P, Nikolov I, Simeonov SP, Iordanova V. Dinamika na tireoidnite khormoni i niakoi khematologichni i biokhimichni pokazateli pri khronichno nitratno otraviane na ovtse. Veterinarnomeditsinski Nauki 1987; 24: 58-62.

Getty R, Hillmann DJ. Equine osteology. In: Getty R, ed. Sisson and Grossman's The Anatomy of the Domestic Animals. Toronto: WB Saunders, 1975: 255-348.

Glade MJ, Luba NK. Serum triiodothyronine and thyroxine concentrations in weanling horses fed carbohydrate by direct gastric infusion. American Journal of Veterinary Research 1987; 48: 578-582.

Glade MJ, Reimers TJ. Effects of dietary energy supply on serum thyroxine, tri-iodothyronine and insulin concentrations in young horses. Journal of Endocrinology 1985; 104: 93-98.

Glade MJ, Gupta S, Reimers TJ. Hormonal responses to high and low planes of nutrition in weanling Thoroughbreds. Journal of Animal Science 1984; 59: 658-665.

Goldberg RC, Chaikoff IL. A simplified procedure for thyroidectomy of the new-born rat without concomitant parathyroidectomy. Endocrinology 1949; 45: 64-70.

Grant DB, Smith I. Survey of neonatal screening for primary hypothyroidism in England, Wales, and Northern Ireland 1982-4. British Medical Journal 1988; 296: 1355-1358.

Grant DB, Smith I, Fuggle PW, Tokar S, Chapple J. Congenital hypothyroidism detected by neonatal screening: relationship between biochemical severity and early clinical features. Archives of the Diseases of Children 1992; 67: 87-90.

Greco DS, Feldman EC, Peterson ME, Turner JL, Hodges CM, Shipman LW. Congenital hypothyroid dwarfism in a family of Giant Schnauzers. Journal of Veterinary Internal Medicine 1991; 5: 57-65.

Greco DS, Peterson ME, Cho DY, Markovits JE. Juvenile-onset hypothyroidism in a dog. Journal of the American Veterinary Medical Association 1985; 187: 948-950.

Greenberg RS, Ibrahim MA. The case-control study. In: Holland WW, Detels R, Knox G, eds. Oxford Textbook of Public Health. 2nd ed. vol 2. Toronto: Oxford University Press, 1991: 121-143.

Gruener N, Shuval HI, Behroozi K, Cohen S, Shechter H. Methemoglobinemia induced by transplacental passage of nitrites in rats. Bulletin of Environmental Contamination and Toxicology 1973; 9: 44-48.

Hamburgh M, Lynn E. The influence of temperature on skeletal maturation of hypothyroid rats. The Anatomical Record 1964; 150: 163-172.

Harris P, Marlin D, Gray J. Equine thyroid function tests: a preliminary investigation. British Veterinary Journal 1992; 148: 71-80.

Held JP, Oliver JW. A sampling protocol for the thyrotropin-stimulation test in the horse. Journal of the American Veterinary Medical Association 1984; 184: 326-327.

Held JP, Patton CS, Toal RL, Geiser DR. Work intolerance in a horse with thyroid carcinoma. Journal of the American Veterinary Medical Association 1985; 187: 1044-1045.

Helrich K, ed. Method #986.31 - Nitrate in forages. Official Methods of Analysis. 15th ed. vol 1. Arlington, Virginia: Association of Official Analytical Chemists, 1990: 357-358.

Hightower D, Miller L, Kyzar JR. Thyroid function tests in veterinary medicine II. Results and applications. Southwestern Veterinarian 1969; 23: 15-21.

Hightower D, Miller L, Kyzar JR. Comparison of serum and plasma thyroxine determinations in horses. Journal of the American Veterinary Medical Association 1971; 159: 449-450.

Hillenbrand L. The take-charge thyroid. Equus. 1991 April: 48-51, 120-123.

Hillyer MH, Taylor GR. Cutaneous manifestations of suspected hypothyroidism in a horse. Equine Veterinary Education 1992; 4: 116-118.

Hines MT, Doles J, Gay C, Schott HC, Cudaback L. Thyroid hyperplasia and musculoskeletal deformity in neonatal foals. Proceedings of the 14th American College of Veterinary Internal Medicine Forum 1996: 544-545.

Hirneth H, Classen HG. Inhibition of nitrate-induced increase of plasma nitrite and methemoglobinemia in rats by simultaneous feeding of ascorbic acid or tocopherol. Arzneimittelforschung 1984; 35: 988-991.

Hood DM, Hightower D, Amoss MS, Williams JD, Gremmel SM, Stephens KA, Stone BA, Grossenbaugh DA. Thyroid function in horses affected with laminitis. Southwestern Veterinarian 1987; 38: 85-91.

Hopkins PS, Thorburn GD. The effect of foetal thyroidectomy on the development of the ovine fetus. Jounal of Endocrinology 1972; 54: 55-66.

Horning H, Ellinger C, Nagel M, Paldy A, Desi I. Zur wirkung von phenylquecksilberacetat und nitrat bei kombinierter verabreichung an ratten: schilddruse, enzyme der leber, morphologische befunde an gehirn und nieren. Nahrung 1986; 30: 713-721.

Ilicki A, Larsson A, Mortensson W. Neonatal skeletal maturation in congenital hypothyroidism and its prognostic value for psychomotor development at 3 years in patients treated early. Hormone Research 1990; 33: 260-264.

Irvine CHG. Thyroid function in the horse. Proceedings of the 12th Annual Convention of the American Association of Equine Practitioners 1966: 197-206.

Irvine CHG. Hypothyroidism in the foal. Equine Veterinary Journal 1984; 16: 302-306.

Irvine CHG, Evans MJ. Postnatal changes in total and free thyroxine and triiodothyronine in foal serum. Journal of Reproduction and Fertility 1975; Supplement 23: 709-715.

Irvine CHG, Evans MJ. Hypothyroidism in foals. New Zealand Veterinary Journal 1977; 25: 354.

Jahreis G, Hesse V, Prange H, Low O. Effect of long-term feeding of nitrate on thyroid function, plasma and tissue concentrations of somatomedin-C/insulin-like growth factor-1 (Sm-C/IGF-1) in male and female rats. Proceedings of the 6th International Trace Element Symposium. vol 3, 1989: 786-793.

Jahreis G, Hesse V, Schone F, Hennig A, Gruhn K. Effect of chronic dietary nitrate and different iodine supply on porcine thyroid function, somatomedin-C level and growth. Experimental and Clinical Endocrinology 1986a; 88: 242-248.

Jahreis G, Hesse V, Schone F, Ludke H, Hennig A, Mehnert E. Einfluss von nitrat und pflanzlichen goitrogenen auf die. Monatshefte fur Veterinarmedizin 1986b; 41: 528-530.

Jahreis G, Schone F, Ludke H, Hesse V. Growth impairment caused by dietary nitrate intake regulated via hypothyroidism and decreased somatomedin. Endocrinologia Experimentalis 1987; 21: 171-180.

Johnson AL. Serum concentrations of prolactin, thyroxine and triiodothyronine relative to season and the estrous cycle in the mare. Journal of Animal Science 1986; 62: 1012-1020.

Johnson JL, Hergert GW, Schneider NR, Grabouski P. Post-harvest change in cornstalk nitrate and its relationship to bovine fetal nitrite/nitrate exposure. In: James LF, Keeler RF, Bailey EM, Cheeke PR, Hegarty MP, eds. Poisonous Plants - Proceedings of the 3rd International Symposium. Ames, Iowa: Iowa State University Press 1992: 423-430.

Johnson JL, Schneider NR, Kelling CL, Doster AR. Nitrate exposure in perinatal beef calves. Proceedings of the 26th Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians 1983: 167-180.

Jones BR, Gruffydd-Jones TJ, Sparkes AH, Lucke VM. Preliminary studies on congenital hypothyroidism in a family of Abyssinian cats. Veterinary Record 1992; 131: 145-148.

Kallfelz FA, Erali RP. Thyroid function tests in domesticated animals: free thyroxine index. American Journal of Veterinary Research 1973; 34: 1449-1451.

Kallfelz FA, Lowe JE. Some normal values of thyroid function in horses. Journal of the American Veterinary Medical Association 1970; 156: 1888-1891.

Kaneko JJ. Thyroid function studies in the horse, in Proceedings. American Association of Equine Practitioners Convention 1964; 10: 125-129.

Katovich M, Evans JW, Sanchez O. Effects of season, pregnancy and lactation on thyroxine turnover in the mare. Journal of Animal Science 1974; 38: 811-818.

Kelly ST, Oehme FW. Circulating thyroid levels in dogs, horses and cattle. Veterinary Medicine and Small Animal Clinician 1974; 69: 1531-1533.

Kelly ST, Oehme FW, Brandt GW. Measurement of thyroid gland function during the estrous cycle of nine mares. American Journal of Veterinary Research 1974; 35: 657-660.

Kerr GR, Tyson IB, Allen JR, Wallace JH, Scheffler G. Deficiency of thyroid hormone and development of the fetal rhesus monkey. Biology of the Neonate 1972; 21: 282-295.

Korber R, Groppel B, Leirer R. Untersuchungen zum jod und schilddrusenstoffwechsel bei kuhen und schafen unter experimenteller nitratbelastung. Spurenelement Symposium 1983; 4: 178-186.

Koterba AM. Prematurity. In: Koterba AM, Drummond WH, Hosch PC, eds. Equine Clinical Neonatology. Philadelphia: Lea & Febiger, 1990: 55-70.

Kreplin C, Allen A. Congenital hypothyroidism in foals in Alberta. Canadian Veterinary Journal 1991; 32: 751.

LaFranchi SH. Hypothyroidism. Pediatric Clinics of North America 1979; 26: 33-51.

Lahti E, Harri M, Lindqvist OV. Uptake and distribution of radioiodine, and the effect of ambient nitrate, in some fish species. Comparative Biochemistry and Physiology A 1985; 80: 337-342.

Larsen PR, Ingbar SH. The thyroid gland. In: Wilson JD, Foster DW, eds. Williams Textbook of Endocrinology. 8th ed. Toronto: WB Saunders, 1992: 357-487.

Lascelles AK, Setchell BP. Hypothyroidism in sheep. Australian Journal of Biological Science 1959; 12: 455-465.

Lee C, Weiss R, Horvath DJ. Effects of nitrogen fertilization on the thyroid function of rats fed 40% orchard grass diets. Journal of Nutrition 1970; 100: 1121-1126.

Letarte J, Garagorri JM. Congenital hypothyroidism: laboratory and clinical investigations of early detected infants. In: Collu R, Ducharme JR, Guyda HJ, eds. Pediatric Endocrinology. New York: Raven Press, 1989: 449-471.

Lokai MD, Ford J. Disorders of endochondral ossification. Equine Practice 1981; 3: 48-52.

Lorey F, Cunningham GC. Birth prevalence of primary congenital hypothyroidism by sex and ethnicity. Human Biology 1992; 64: 531-538.

Lothrop CD, Nolan HL. Equine thyroid function assessment with the thyrotropin-releasing hormone response test. American Journal of Veterinary Research 1986; 47: 942-944.

Lowe JE, Baldwin BH, Foote RH, Hillman RB, Kallfelz FA. Equine hypothyroidism: the long term effects of thyroidectomy on metabolism and growth in mares and stallions. Cornell Veterinarian 1974; 64: 276-295.

Lowe JE, Baldwin BH, Foote RH, Hillman RB, Kallfelz FA. Semen characteristics in thyroidectomized stallions. Journal of Reproduction and Fertility 1975; Supplement 23: 81-86.

Lowe JE, Foote RH, Baldwin BH, Hillman RB, Kallfelz FA. Reproductive patterns in cyclic and pregnant thyroidectomized mares. Journal of Reproduction and Fertility 1987; Supplement 35: 281-288.

Lowe JE, Kallfelz FA. Thyroidectomy and the T-4 test to assess thyroid dysfunction in the horse and pony. Proceedings of the 16th Annual Convention of the American Association of Equine Practitioners 1970; 16: 135-154.

Lusted LB, Pickering DE, Fisher D, Smyth FC. Growth and metabolism in normal and thyroid-ablated infant rhesus monkeys (Macaca mulatta). American Journal of the Diseases of Children 1953; 86: 426-435.

Malestein A, Geurink JH, Schuyt G, Schotman AJH, Kemp A, van't Klooster AT. Nitrate poisoning in cattle. 4. The effect of nitrate dosing during parturition on the oxygen capacity of maternal blood and the oxygen supply to the unborn calf. Veterinary Quarterly 1980; 2: 149-159.

Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. Journal of the National Cancer Institute 1959; 22: 719-748.

Martin SW, Meek AH, Willeberg P. Veterinary Epidemiology: Principles and Methods. Ames: Iowa State University Press, 1987: 146-175.

McBride GE, Christopherson RJ, Sauer W. Metabolic rate and plasma thyroid hormone concentrations of mature horses in response to changes in ambient temperature. Canadian Journal of Animal Science 1985; 65: 375-382.

McLaughlin BG, Doige CE. Congenital musculoskeletal lesions and hyperplastic goitre in foals. Canadian Veterinary Journal 1981; 22: 130-133.

McLaughlin BG, Doige CE. A study of carpal and tarsal bones in normal and hypothyroid foals. Canadian Veterinary Journal 1982; 23: 164-168.

McLaughlin BG, Doige CE, Fretz PB, Pharr JW. Carpal bone lesions associated with angular limb deformities in foals. Journal of the American Medical Association 1981; 178: 224-230.

McLaughlin BG, Doige CE, McLaughlin PS. Thyroid hormone levels in foals with congenital musculoskeletal lesions. Canadian Veterinary Journal 1986; 27: 264-267.

Medleau L, Eigenmann JE, Saunders HM, Goldschmidt MH. Congenital hypothyroidism in a dog. Journal of the American Animal Hospital Association 1985; 21: 341-344.

Messer NT. Clinical and diagnostic features of thyroid disease in horses. Proceedings of the 11th Annual American College of Veterinary Internal Medicine Forum 1993: 649-651.

Messer NT. Thyroid Dysfunction in Horses. Proceedings of the 40th Annual Convention of the American Association of Equine Practitioners 1994: 153-154.

Messer NT, Ganjam VK, Nachreiner RF, Krause GF. Effects of dexamethasone administration on serum thyroid hormone concentrations in clinically normal horses. Journal of the American Veterinary Medical Association 1995a; 206: 63-66.

Messer NT, Johnson PJ, Refsal KR, Nachreiner RF, Ganjam VK, Krause GF. Effect of food deprivation on baseline iodothyronine and cortisol concentrations in healthy, adult horses. American Journal of Veterinary Research 1995b; 56: 116-121.

Miyazawa K, Motoyoshi S, Usui K. Nodular goiters of three mares and their foals, induced by feeding excessive amount of seaweed. Japanese Journal of Veterinary Science 1978; 40: 749-753.

Mooney CT, Anderson TJ. Congenital hypothyroidism in a boxer dog. Journal of Small Animal Practice 1995; 34: 31-35.

Morris DD, Garcia M. Thyroid stimulating hormone: response test in healthy horses, and effect of phenylbutazone on equine thyroid hormones. American Journal of Veterinary Research 1983; 44: 503-507.

Morris DD, Garcia M. Effects of phenylbutazone and anabolic steroids on adrenal and thyroid gland function tests in healthy horses. American Journal of Veterinary Research 1985; 46: 359-364.

Murphy BD, Mapletoft RJ, Manns J, Humphrey WD. Variability in gonadotrophin preparations as a factor in the superovulatory response. Theriogenology 1984; 21: 117-125.

Murray MJ, Luba NK. Plasma gastrin and somatostatin, and serum thyroxine (T_4), triiodothyronine ($_{T3}$), reverse triiodothyronine (r_{T3}) and cortisol concentrations in foals from birth to 28 days of age. Equine Veterinary Journal 1993; 25: 237-239.

Myers VS, Gordon GW. Ruptured common digital extensor tendons associated with contracted flexor tendons in foals. Proceedings or the 21st Annual Convention of the American Association of Equine Practitioners 1975; 21: 67-74.

Nachreiner RF, Hyland JH. Reproductive endocrine function testing in mares. In: McKinnon AO, Voss JL, eds. Equine Reproduction. Philadelphia: Lea & Febiger, 1993: 303-310.

National Research Council. Nutrient Requirements of Horses, 5th ed. Washington, DC: National Academy Press, 1989: 16.

National Research Council Committee on Nitrate Accumulation. Accumulation of Nitrate. Washington, DC: National Academy of Sciences, 1972.

Nathanielsz PW. Fetal Endocrinology: An Experimental Approach. Amsterdam: North-Holland, 1976: 53-72 and 73-87. (Monographs in Fetal Physiology, volume 1).

Neilson FJA. Nitrite and nitrate poisoning with special reference to 'grasslands tama' ryegrass. New Zealand Veterinary Journal 1974; 22: 12-13.

Newcomb R, Christie WB, Rowson LEA, Walters DE, Bousfield WED. Influence of dose, repeated treatment and batch of hormone on ovarian response in heifers treated with PMSG. Journal of Reproduction and Fertility 1979; 56: 113-118.

Newland CJ, Swift PGF, Lamont AC. Congenital hypothyroidism - correlation between radiographic appearances of the knee epiphyses and biochemical data. Postgraduate Medical Journal 1991; 67: 553-556.

Newsom IE, Stout EN, Thorp F, Barber CW, Groth AH. Oat hay poisoning. Journal of the American Veterinary Medical Association 1937; 90: 66-75.

Nicholas FW. Introduction to Veterinary Genetics. New York: Oxford University Press, 1996: 155-161

Noback CR, Barnett JC, Kupperman HS. The time of appearance of ossification centers in the rat as influenced by injections of thyroxin, thyiouracil, estradiol and testosterone propionate. The Anatomical Record 1949; 103: 49-68.

Nye L, Yeo TH, Chan V, Goldie D, Landon J. Stability of thyroxine and triiodothyronine in biological fluids. Journal of Clinical Pathology 1975; 28: 915-919.

O'Hara PJ, Fraser AJ. Nitrate poisoning in cattle grazing crops. New Zealand Veterinary Journal 1975; 23: 45-53.

Oliver JW, Held JP. Thyrotropin stimulation test - new perspective on value of monitoring triiodothyronine. Journal of the American Veterinary Medical Association 1985; 187: 931-934.

Olson LC, Palotay JL, Haines JE, Hanada J, Bergquist DY. Compensated, goitrous hypothyroidism in rhesus macaques. Laboratory Animal Science 1985; 35: 629-634.

Osweiler GD, Carson TL, Buck WB, Van Gelder GA. Clinical and Diagnostic Veterinary Toxicology, 3rd ed. Dubuque, Iowa: Kendall/Hunt, 1985: 460-467.

Peterson ME, Ferguson DC. Thyroid diseases. In: Ettinger SJ, ed. Textbook of Veterinary Internal Medicine. 3rd ed. vol 2. Philadelphia: WB Saunders, 1989: 1632-1675.

Pethes G, Korber R, Gurtler H, Furcht G, Zastrow HJ. Konzentration an butanolextrahierbarem jod und thyroxin im blutplasma von kalbern mit und ohne kropfbildung und deren muttertieren. Monatshefte fur Veterinarmedizin 1983; 38: 567-571.

Pharr JW, Fretz PB. Radiographic findings in foals with angular limb deformities. Journal of the American Veterinary Medical Association 1981; 179: 812-817.

Pool RR. Difficulties in definition of equine osteochondrosis; differentiation of developmental and acquired lesions. Equine Veterinary Journal 1993; Supplement 16: 5-12.

Prasad J. Effect of high nitrate diet on thyroid glands in goats. Indian Journal of Animal Science 1983; 53: 791-794.

Putnam MR, Bransby DI, Schumacher J, Boosinger TR, Bush L, Shelby RA, Vaughan JT, Ball D, Brendemuehl JP. Effects of the fungal endophyte Acremonium coenophialum in fescue on pregnant mares and foal viability. American Journal of Veterinary Research 1991; 52: 2071-2074.

Ray RD, Asling CW, Walker DG, Simpson ME, Li CH, Evans HM. Growth and differentiation of the skeleton in thyroidectomized-hypophysectomized rats treated with thyroxin, growth hormone and the combination. The Journal of Bone and Joint Surgery 1954; 36: 94-103.

Ray RD, Simpson ME, Li CH, Asling CW, Evans HM. Effects of the pituitary growth hormone and of thyroxin on growth and differentiation of the rat thyroidectomized at birth. American Journal of Anatomy 1950; 86: 479-516.

Reap M, Cass C, Hightower D. Thyroxine and triiodothyronine levels in ten species of animals. Southwestern Veterinarian 1978; 31: 31-34.

Reimers TJ, Cowan BA, Davidson HP, Colby ED. Validation of radioimmunoassays for triiodothyronine, thyroxine, and hydrocortisone (cortisol) in canine, feline, and equine sera. American Journal of Veterinary Research 1981; 42: 2016-2021.

Reimers TJ, McCann JP, Cowan RG, Concannon PW. Effects of storage, hemolysis, and freezing on concentrations of thyroxine, cortisol and insulin in blood samples. Proceedings of the Society for Experimental Biology and Medicine 1982; 170: 509-516.

Reimers TJ, McCann JP, Cowan RG. Effects of storage times and temperatures on T_3, T_4, LH, prolactin, insulin, cortisol and progesterone concentrations in blood samples from cows. Journal of Animal Science 1983; 57: 683-691.

Reimers TJ, Lamb SV, Bartlett SA, Matamoros RA, Cowan RG, Engle JS. Effects of hemolysis and storage on quantification of hormones in blood samples from dogs, cattle, and horses. American Journal of Veterinary Research 1991; 52: 1075-1080.

Ridder WE, Oehme FW. Nitrates as an environmental, animal, and human hazard. Clinical Toxicology 1974; 7: 145-159.

Riggs CW. Nitrite poisoning from ingestion of plants high in nitrate. American Journal of Veterinary Research 1945; 6: 194-197.

Roberts SJ. Veterinary Obstetrics and Genital Diseases. Ithaca, New York: by the author, 1971: 27.

Roberts SJ. Gestation and pregnancy diagnosis in the mare. In: Morrow DA, ed. Current Therapy in Theriogenology, 2nd ed. Toronto: WB Saunders, 1986: 670-678.

Robinson WF, Shaw SE, Stanley B, Wyburn RS. Congenital hypothyroidism in Scottish Deerhound puppies. Australian Veterinary Journal 1988; 65: 386-389.

Rodenwold BW, Simms BT. Iodine for brood mares. Record of Proceedings of the 27th Annual Meeting of the American Society of Animal Production 1934: 89-92.

Rooney JR. Disease of bone. In: Catcott EJ, Smithcors JF, eds. Equine Medicine and Surgery. 2nd ed. Wheaton, Illinois: American Veterinary Publications, 1972: 489-501

Rothman KJ. Modern Epidemiology. Toronto: Little, Brown and Company, 1986: 51-76.

Ruohoniemi M. Use of ultrasonography to evaluate the degree of ossification of the small tarsal bones in 10 foals. Equine Veterinary Journal 1993; 25: 539-543.

Sauerbrey G, Andree B, Kunze M, Mey W. Untersuchungen uber die endemische struma und ihre beziehung zu verschiedenen trinkwasser faktoren in 4 gemeinden des bezirkes Suhl. Zeitschrift fuer die Gesamte Innere Medizin 1989; 44: 267-270.

Saunders HM, Jezyk PK. The radiographic appearance of canine congenital hypothyroidism: Skeletal changes with delayed treatment. Veterinary Radiology 1991; 32: 171-177.

Schlesselman JJ, Stolley PD. Research strategies. In: Schlesselman JJ, ed. Case-Control Studies: Design, Conduct, Analysis. New York: Oxford University Press 1982: 7-26.

Schlotthauer CF. The incidence and types of disease of the thyroid gland of adult horses. Journal of the American Veterinary Medical Association 1931; 78: 211-218.

Scow RO, Simpson ME. Thyroidectomy in the newborn rat. The Anatomical Record 1945; 91: 209-225.

Setchell BP, Dickinson DA, Lascelles AK, Bonner RB. Neonatal mortality in lambs associated with goitre. Australian Veterinary Journal 1960; 36: 159-164.

Shaftoe S, Pichler-Schick M, Chen CL. Thyroid stimulating hormone response tests in one-day-old foals. Journal of Equine Veterinary Science 1988; 8: 310-312.

Shaver JR, Fretz PB, Doige CE, Williams DM. Skeletal manifestations of suspected hypothyroidism in two foals. Journal of Equine Medicine and Surgery 1979; 3: 269-275.

Shrader RE, Joy N, Zeman FJ. Fetal ossification site development in induced hypothyroidism in rats. Nutrition Reports International 1979; 20: 403-410.

Silva CAM, Merkt H, Bergamo PNL, Barros SS, Barros CSL, Santos MN, Hoppen HO, Heidemann P, Meyer H. Consequences of excess iodine supply in a Thoroughbred stud in southern Brazil. Journal of Reproduction and Fertility 1987; Supplement 35: 529-533.

Silver M, Ousey JC, Dudan FE, Fowden AL, Knox J, Cash RS, Rossdale PD. Studies on equine prematurity 2: Post natal adrenocortical activity in relation to plasma adrenocorticotropic hormone and catecholamine levels in term and premature foals. Equine Veterinary Journal 1984; 16: 278-286

Silver M, Fowden AL, Knox J, Ousey J, Cash R, Rossdale PD. Relationship between circulating tri-iodothyronine and cortisol in the perinatal period in the foal. Journal of Reproduction and Fertility 1991; Supplement 44: 619-626.

Silverman FN. Bone changes in systemic body disorders. In: Silverman FN, Kuhn JP, eds. Caffey's Pediatric X-ray Diagnosis: An Integrated Imaging Approach. 9th ed. St. Louis: Mosby-Year Book, 1993: 1911-1934.

Sinha DP, Sleight SD. Pathogenesis of abortion in acute nitrite toxicosis in guinea pigs. Toxicology and Applied Pharmacology 1971; 18: 340-347.

Sippel WL. A veterinarian's approach to stud farm nutrition. Equine Veterinary Journal 1968; 1: 203-211.

Sisson S. Equine syndesmology. In: Getty R, ed. Sisson and Grossman's The Anatomy of the Domestic Animals. 5th ed. vol 1. Toronto: Saunders, 1975: 349-375.

Sjollema BE, den Hartog MT, de Vijlder JJM, van Dijk JE, Rijnberk A. Congenital hypothyroidism in two cats due to defective organification: data suggesting loosely anchored thyroperoxidase. Acta Endocrinologia 1991; 125: 435-440.

Slanina L, Slivka P, Struharikova R. Transmammarny prestup dusicnanov a dusitanov u prezuvavcov a uroven methemoglobinemie v krvi mladat a ich matiek. Veterinari Medicina (Praha) 1990; 35: 647-656.

Smith CA. Is your horse hypothyroid? Horse Illustrated. 1994 January: 32-35.

Smith RA, Suleiman A. Nitrite intoxication from large round bales. Veterinary and Human Toxicology 1991; 33: 349-350.

Smith RD. Veterinary Clinical Epidemiology. Toronto: Butterworth-Heinemann, 1991: 13-28, 29-43.

Smythe RH. Diseases of the endocrine system. In: Bone JF, Catcott EJ, Gabel AA, Johnson LE, Riley WF, eds. Equine Medicine and Surgery. 1st ed. Wheaton, Illinois: American Veterinary Publications, 1963: 291-294.

Sojka J. Factors which affect serum T_3 and T_4 levels in the horse. Equine Practice 1993; 15: 15-19.

Sojka JE. Thyroid Evaluation in Horses. Proceedings of the 14th American College of Veterinary Internal Medicine Forum 1996: 546.

Sojka JE, Levy M. Evaluation of endocrine function. Veterinary Clinics of North America: Equine Practice 1995; 11: 415-435.

Sojka JE, Johnson MA, Bottoms GD. Serum triiodothyronine, total thyroxine, and free thyroxine concentrations in horses. American Journal of Veterinary Research 1993; 54: 52-55.

Spring T, Hofler J, Meyer J, Huchzermeier R, Abano D, Fino J, Flentge C, Kirkemo C, Shipchandler M, Vaughan K. The development of fluorescence polarization assays for serum thyroxine and unsaturated thyroxine binding proteins. In: Proceedings of the 13th Annual Meeting of the European Thyroid Association. Madrid: Annales d'endocrinologie 1983; 44: 64A.

Stanley O, Hillidge CJ. Alopecia associated with hypothyroidism in a horse. Equine Veterinary Journal 1982; 14: 165-167.

Sticker LS, Thompson DL, Fernandez JM, Bunting LD, Depew CL. Dietary protein and(or) energy restriction in mares: plasma growth hormone, IGF-I, prolactin, cortisol, and thyroid hormone responses to feeding, glucose, and epinephrine. Journal of Animal Science 1995; 73: 1424-1432.

Štrbák V, Tomšík F. Thyroid hormone levels in cow maternal and fetal sera during last trimester of pregnancy. Endocrinologia Experimentalis 1988; 22: 113-116.

Styne DM. Endocrine disorders. In: Behrman RE, Kliegman RM, eds. Nelson Essentials of Pediatrics. 2nd ed. Toronto: WB Saunders, 1994: 611-655.

Symons RG, Vining RF. An Evaluation of a Fluorescence Polarization Immunoassay of Thyroxin and Thyroxin-Uptake. Clinical Chemistry 1985; 31: 1342-1348.

Takagi S, Ito K, Shibata H. Effects of training on plasma fibrinogen concentrations and thyroid hormone level in young racehorses. Experimental Reports of the Equine Health Laboratory 1974; 11: 94-105.

Tanase H, Kudo K, Horikoshi H, Mizushima H, Okazaki T, Ogata E. Inherited primary hypothyroidism with thyrotropin resistance in Japanese cats. Journal of Endocrinology 1991; 129: 245-251.

Thomas CL, Adams JC. Radioimmunoassay of equine serum for thyroxine: reference values. American Journal of Veterinary Research 1978; 39: 1239.

Thomas AL, Nathanielsz PW. The fetal thyroid. In: Martini L, James VHT, eds. Fetal Endocrinology and Metabolism. New York: Academic Press, 1983: 97-116. (Current Topics in Experimental Endocrinology, volume 5).

Thomas DC, Siemiatycki J, Dewar R, Robins J, Goldberg M, Armstrong BG. The problem of multiple inference in studies designed to generate hypotheses. American Journal of Epidemiology 1985; 122: 1080-1095.

Thompson FN, Caudle AB, Kemppainen RJ, Nett TM, Brown J, Willians DJ. Thyroidal and prolactin secretion in agalactic mares. Theriogenology 1986; 25: 575-580.

Thorburn GD, Hopkins PS. Thyroid function in the foetal lamb. In: Cromline KS, Cross KW, Dawes GS, Nathanielsz PW, eds. Foetal and Neonatal Physiology: Proceedings of the Sir Joseph Barcroft Centenary Symposium. London: Cambridge University Press, 1973: 488-496.

Thorp F. Further observations on oat hay poisoning. Journal of the American Veterinary Medical Association 1938; 92: 159-170.

Toublanc JE. Comparison of epidemiological data on congenital hypothyroidism in Europe with those of other parts in the world. Hormone Research 1992; 38: 230-235.

Turner AS, Fretz PB. A comparison of surgical techniques and associated complications of transphyseal bridging in foals. Proceedings of the 23rd Convention of the American Association of Equine Practitioners 1977: 275-303.

Ubom GA. The goitre-soil-water-diet relationship: case study in Plateau State, Nigeria. Science of the Total Environment 1991; 107: 1-11.

Utiger RD. Decreased extrathyroidal triiodothyronine production in nonthyroidal illness: Benefit or harm? American Journal of Medicine 1980; 69: 807-810.

Vandeplassche M. Dystocia. In: McKinnon AO, Voss JL, eds. Equine Reproduction. Philadelphia: Lea & Febiger, 1993: 578-587.

Vivrette SL, Reimers TJ, Krook L. Skeletal disease in a hypothyroid foal. Cornell Veterinarian 1984; 74: 373-386.

Waldron-Mease E. Hypothyroidism and myopathy in racing Thoroughbreds and Standardbreds. Journal of Equine Medicine and Surgery 1979; 3: 124-128.

Wartofsky L, Burman KD. Alterations in thyroid function in patients with systemic illness: The "euthyroid sick syndrome". Endocrine Reviews 1982; 3: 164-217.

Weiss RM, Noback CR. The effects of thyroxin and thiouracil on the time of appearance of ossification centers of rat fetuses. Endocrinology 1949; 45: 389-395.

Welsch CW, Bloomfield RA, Garner GB, Muhrer ME. Effect of dietary nitrate on thyroid and adrenal gland weight. Journal of Animal Science 1961; 20: 981.

Welsch CW, Bloomfield RA, Garner GB, Muhrer ME. Response of rats to low temperature and nitrate. Journal of Animal Science 1962; 21: 1032.

Wilkins L. Epiphyseal dysgenesis associated with hypothyroidism. American Journal of Diseases of Children 1941; 61: 13-34.

Wilson RB, Dickson WM, Frost FN. A procedure for assay of thyroid status in animals. Veterinary Medicine 1961; 56: 285-289.

Wright MJ, Davison KL. Nitrate accumulation in crops and nitrate poisoning in animals. Advances in Agronomy 1964; 16: 197-247.

Wyngaarden JB, Stanbury JB, Rapp B. The effects of iodine, perchlorate, thiocyanate, and nitrate administration upon the iodine concentrating mechanism of the rat thyroid. Endocrinology 1953; 52: 568-574.

Wyngaarden JB, Wright BM, Ways P. The effect of certain anions upon the accumulation and retention of iodine by the thyroid gland. Endocrinology 1952; 50: 537-549.

Yadav KP, Garner GB, O'Dell BL, Flynn LM, Bloomfield RA, Muhrer ME. Iodine and nitrate as factors in vitamin A storage. Journal of Animal Science 1962; 21: 1017.

Yalow RS. Radioimmunoassay of hormones. In: Wilson JD, Foster DW, eds. Williams Textbook of Endocrinology. 8th ed. Toronto: WB Saunders, 1992: 1635-1645.

Yong CW, Griffin S. Goiter in foals on a pregnant mare urine farm. Canadian Veterinary Journal 1992; 33: 276-277.

Youngquist RS. Equine obstetrics. In: Morrow DA, ed. Current Therapy in Theriogenology, 2nd ed. Toronto: WB Saunders, 1986: 693-699.

Zerbe CA, Padgett RF, Nachriener RF, Schall WD. Congenital hypothyroidism in the Scottish Deerhound. Proceedings of the 6th American College of Veterinary Internal Medicine Forum 1988: 721.

APPENDICES

Appendix A. Summary of reported triiodothyronine concentrations in the serum or plasma of horses 1 year of age and older

Number and Breed; Sex	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
100 TB and STD; unspecified	adults	untrained, non-pregnant	RIA	0.0154	1.2		Irvine 1975
54 TB; S,G,M	≥ 1 y		RIA	none	1.2	0.3 - 2.0[1]	Blackmore 1978
9 TB; M	unspecified	pregnant	RIA	none	1.2	0.6 - 2.0[1]	Blackmore 1978
34 TB; unspecified	racing age	multiple samples over 5 mo	RIA	none	1.7	0.6 - 4.8[1]	Blackmore 1978
18 TB; S,G,M	2 or 3 y	6 horses included above	RIA	none	1.3	0.8 - 2.5[1]	Blackmore 1978
5 unspecified; S	unspecified		RIA	0.0154	1.7	1.0 - 2.4[1]	Reap 1978
5 unspecified; M	unspecified		RIA	0.0154	0.6	0.5 - 1.0[1]	Reap 1978
38 various; S,G,M	2 to 25 y		RIA	0.0154	1.5[2]	0.7 - 2.8[1]	Chen 1981
35 unspecified; S,G,M	3 to 15 y		RIA	1.54	1.2[2]	0.4 - 2.0[3,4]	Morris 1983
11 unspecified; S,G,M	3 to 15 y		RIA	1.54	1.0[2]	0.0 - 2.0[3,4]	Morris 1983

Appendix A. (continued)

Summary of reported triiodothyronine concentrations in the serum or plasma of horses 1 year of age and older

Number and Breed; Sex	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
5 light breed; M	4 to 18 y		RIA	0.0154	0.8		Chen 1984
59 unspecified; unspecified	adults		RIA	none	1.2	0.9 - 1.6[4]	Irvine 1984
12 unspecified; S,G,M	3 to 21 y		RIA	1.54	1.2	0.6 - 1.8[4]	Beech 1985
12 TB; M	4 to 15 y	multiple samples per horse	RIA	0.0154	1.2[2,5]		Chen 1985
12 TB; unspecified	12 to 14 mo		RIA	none	1.1[2,5]		Glade 1985
6 QH; G	mature	ambient temperatures <0°C	RIA	0.0154	1.1[2]	0.4 - 1.8[4,6]	McBride 1985
12 unspecified; M	3 to 12 y		RIA	1.54	1.2	0.3 - 2.1[4]	Morris 1985
10 various; G, M	mature		RIA	1.54	0.6	0.3 - 0.9[4]	Oliver 1985
31 STD and TB; unspecified	2 to 9 y	in training	RIA	1.54	1.5	-0.1 - 3.2[4,6]	Garcia 1986

Appendix A. (continued)

Summary of reported triiodothyronine concentrations in the serum or plasma of horses 1 year of age and older

Number and Breed; Sex	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
17 STD and TB; unspecified	2 to 9 y	in training	RIA	1.54	1.5	0.3 - 2.8[4,6]	Garcia 1986
6 STD; M	5 to 17 y	sampled monthly for 1 y	RIA	1.54		0.2 - 0.9[5,7]	Johnson 1986
11 various; S,G,M	adults	5 horses and 6 ponies	RIA	1.54	0.7	0.1 - 1.2[4,6]	Lothrop 1986
8 unspecified; M		post-partum and lactating	RIA	0.0154	0.9		Thompson 1986
21 unspecified; unspecified	adults		RIA	0.0154	0.6	-0.2 - 13[4]	Hood 1987
16 STD; foaling M	4 to 20 y	sampled 4 times over 45 d	ELISA	1.54		1.5 - 2.0[5,7]	Ferlazzo 1988
10 various; G	2 to 13 y	daylight samples[8] over 3 d	RIA	0.0154	0.8[2]	0.4 - 1.2[3]	Duckett 1989
10 various; G	2 to 13 y	night samples[9] over 3 d	RIA	0.0154	0.6[2]	0.3 - 0.9[3]	Duckett 1989
8 STD; M	4 to 12 y	4 pregnant; followed for 1 y	RIA	1.54		0.3 - 1.5[7]	F-B[10] 1991

Appendix A. (continued)

Summary of reported triiodothyronine concentrations in the serum or plasma of horses 1 year of age and older

Number and Breed; Sex	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
4 unspecified; unspecified	unspecified		nr	none	1.0		Hillyer 1992
12 various; 2S, 10M	6 to 19 yr	sampled twice daily for 4 d	RIA	1.54	0.6	0.3 - 1.2[1]	Sojka 1993
12 various; 7G, 5M	2 to 16 y		RIA	none	0.8[2]	-0.2 - 1.9[3,4]	Messer 1985
6 various; 3G, 3M	4 to 16 y		RIA	none	1.0	0.3 - 1.1[4,6]	Messer 1995
16 light breed; M	8 to 9 y	average levels over 25 d	RIA	1.54	1.4	1.1 - 1.6[4,6]	Sticker 1995

CF = factor used to convert from conventional units to Système International d'Unitès
*Single author or first of multiple authors, and date of publication
TB = Thoroughbred, STD = Standardbred horse, QH = Quarter horse
S = stallion(s), G = gelding(s), M = mare(s)
d = day(s), mo = month(s), y = year(s)
CPB = competitive protein binding, RIA = radioimmunoassay, ELISA = enzyme-linked immunosorbent assay, nr = not reported
[1]Minimum and maximum values reported

Appendix A. (continued)

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[2]Mean of two or more reported means
[3]Mean of two or more reported standard deviations
[4]± 1.96 standard deviations from the mean
[5]Values estimated from a graph
[6]Standard deviation calculated from the reported standard error of the mean
[7]Minimum and maximum mean values reported
[8]8:00, 12:00 and 16:00 hours
[9]20:00, 24:00 and 4:00 hours
[10]F-B = Flisińska-Bojanowska

Appendix B. Summary of reported thyroxine concentrations in the serum or plasma of horses 1 year of age and older

Number and Breed; Sex	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
10 unspecified; unspecified	unspecified	6 animals sampled twice	CPB	12.87	20.5	8.4 - 33.5[1]	Hightower 1969
8 STD; unspecified	12 to 15 mo		CPB	12.87	23.7	11.5 - 35.9[2]	Lowe 1970
12 STD; unspecified	15 to 18 mo		CPB	12.87	36.6	14.6 - 58.6[2]	Lowe 1970
6 STD; unspecified	18 to 21 mo		CPB	12.87	31.7	16.6 - 47.8[2]	Lowe 1970
20 STD; unspecified	1 to 3 y	in training	CPB	12.87	21.2	10.0 - 32.4[2]	Lowe 1970
15 STD; unspecified	4 to 6 y	in training	CPB	12.87	20.2	7.7 - 32.7[2]	Lowe 1970
12 STD; unspecified	7 to 10 y	in training	CPB	12.87	13.0	4.4 - 21.6[2]	Lowe 1970
10 QH; 9M, 1S	unspecified		CPB	12.87	31.7	10.7 - 52.7[2]	Hightower 1971
15 QH; M	unspecified		CPB	12.87	21.6	3.4 - 39.8[2]	Hightower 1971
5 unspecified; unspecified	6 to 11 y		CPB	12.87	20.3	-1.8 - 42.4[2]	Kallfelz 1973

Appendix B. (continued)

Summary of reported thyroxine concentrations in the serum or plasma of horses 1 year of age and older

Number and Breed; Sex	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
5 TB and QH; M	4 to 6 y	3.3°C ambient temperature	CPB	1.287	48.6	39.6 - 57.6[2,3]	Katovich 1974
5 TB or QH; M	4 to 6 y	16.6°C ambient temperature	CPB	1.287	28.8	16.5 - 41.1[2,3]	Katovich 1974
6 TB or QH; M	4 to 6 y	21.6°C ambient temperature	CPB	1.287	29.7	2.5 - 56.9[2,3]	Katovich 1974
5 TB or QH; M	5 to 17 y	119 ± 20 d pregnant	CPB	1.287	19.7	1.7 - 37.7[2,3]	Katovich 1974
5 TB or QH; M	6 to 11 y	306 ± 12 d pregnant	CPB	1.287	25.7	23.3 - 28.1[2,3]	Katovich 1974
5 TB or QH; M	6 to 11 y	lactating for 80 ± 12 d	CPB	1.287	21.2	2.0 - 40.4[2,3]	Katovich 1974
3 TB and 1 QH: M	aged	anestrus	CPB	12.87	33.2	18.1 - 55.3[1]	Kelly 1974a
9 ARAB or QH-type; M	> 5 y	several samples per mare	CPB	12.87	18.9	-2.3 - 40.1[2]	Kelly 1974b
6 mixed breed; S	17 to 20 mo		CPB	12.87	35.4[4]	16.9 -53.9[2,3,4,5]	Lowe 1974

Appendix B. (continued)

Summary of reported thyroxine concentrations in the serum or plasma of horses 1 year of age and older

Number and Breed; Sex	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
5 mixed breed; M	17 to 20 mo		CPB	12.87	35.4[4]	24.1 - 46.7[2,3,4,5]	Lowe 1974
39 TB or Anglo-Arab.; both	2 to 3 y	early in training program	CPB	12.87	27.7	- 10.1 - 65.5[2,3]	Takagi 1974
39 TB or Anglo-Arab.; both	2 to 3 y	middle of training program	CPB	12.87	22.4	- 18.6 - 63.4[2,3]	Takagi 1974
39 TB or Anglo-Arab.; both	2 to 3 y	end of training program	CPB	12.87	34.9	- 1.3 - 71.1[2,3]	Takagi 1974
100 TB and STD	adults	untrained, non-pregnant	CPB	12.87	34.8		Irvine 1975
40 TB; S,G,M	2 y		RIA	none	20.7[5]	5 - 35[1]	Blackmore 1978
19 TB; S,G,M	3 y		RIA	none	16.3[5]	5 - 28[1]	Blackmore 1978
4 TB; S	4 y		RIA	none	11		Blackmore 1978
4 TB; G	> 4 y		RIA	none	22		Blackmore 1978

Appendix B. (continued)

Summary of reported thyroxine concentrations in the serum or plasma of horses 1 year of age and older

Number and Breed; Sex	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
9 TB; M	unspecified	pregnant	RIA	none	31.3	3 - 56[1]	Blackmore 1978
34 TB; unspecified	racing age	multiple samples over 5 mo	RIA	none	12.9[6]	<5.0 - 80.0[5]	Blackmore 1978
18 TB; S,G,M	2 or 3 y	6 horses included above	RIA	none	15.0[6]	<5.0 - 35.0[5]	Blackmore 1978
5 unspecified; S	unspecified		RIA	12.87	25.7[2]	19.9 - 30.6[1]	Reap 1978
5 unspecified; M	unspecified		RIA	12.87	16.2[2]	12.2 - 21.2[1]	Reap 1978
157 various; S,G,M	> 1 y		RIA	12.87	20.2	3.9 - 47.6[1]	Thomas 1978
38 various; S,G,M	2 to 25 y		RIA	12.87	22.4[5]	11.6 - 37.3[1]	Chen 1981
35 unspecified; S,G,M	3 to 15 y		RIA	1.287	21.6[5]	5.2 - 38.0[2,7]	Morris 1983
11 unspecified, S,G,M	3 to 15 y		RIA	1.287	28.2[5]	7.6 - 48.8[2,7]	Morris 1983

Appendix B. (continued)

Summary of reported thyroxine concentrations the serum or plasma of horses 1 year of age and older

Number and Breed; Sex	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
5 light breed; M	4 to 18 y		RIA	12.87	19.3		Chen 1984
10 various; both	mature		RIA	1.287	27.4	9.0 - 47.6[1]	Held 1984
31 unspecified; unspecified	adults		RIA	none	22	12.2 - 31.8[2]	Irvine 1984
12 unspecified; S,G,M	3 to 21 y		RIA	1.287	29.5	28.7 - 30.2[2]	Beech 1985
12 TB; M	4 to 15 y	multiple samples per horse	RIA	12.87	23.1[4,5]		Chen 1985
12 TB; unspecified	12 to 14 mo		RIA	none	18.0[4,5]		Glade 1985
6 QH; G	mature	ambient temperatures <0°C	RIA	12.87	23.4[5]	17.2 - 29.6[2,3]	McBride 1985
12 unspecified; M	3 to 12 y		RIA	1.287	30.9	11.6 - 50.2[2]	Morris 1985
10 various; G,M	mature		RIA	1.287	28.3	5.6 - 51.0[2]	Oliver 1985

Appendix B. (continued)

Summary of reported thyroxine concentrations in the serum or plasma of horses 1 year of age and older

Number and Breed; Sex	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
31 STD and TB; unspecified	2 to 9 y	in training	RIA	1.287	29.6	11.3 - 47.9[3]	Garcia 1986
6 STD; M	5 to 17 y	sampled monthly for 1 y	RIA	1.287		17.7 - 24.1[4,8]	Johnson 1986
11 various; S,G,M	adults	5 horses and 6 ponies	RIA	1.287	31.4	9.6 - 53.3[2,3]	Lothrop 1986
8 unspecified; M	unspecified	post-partum and lactating	RIA	12.87	24.1		Thompson 1986
17 STD and TB; unspecified	2 to 9 y	same horses as above	RIA	1.287	28.4	12.8 - 44.0[3]	Garcia 1986
21 unspecified; unspecified	adults		RIA	12.87	19.8	5.2 - 34.5[2]	Hood 1987
2 QH; M	1.5 y		RIA	1.287		12.9 - 38.6[1]	Lowe 1987
16 STD; M	4 to 20 y	15 days prior to foaling	ELISA	12.87		25 - 30[4,8]	Ferlazzo 1988
10 various; G	2 to 13 y	daylight samples[9] over 3 d	RIA	12.87	29.6[5]	11.3 - 47.9[2,7]	Duckett 1989

Appendix B. (continued)

Summary of reported thyroxine concentrations in the serum or plasma of horses 1 year of age and older

Number and Breed; Sex	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
10 various; G	2 to 13 y	night samples[10] over 3 days	RIA	12.87	25.3[5]	6.9 - 43.6[2,7]	Duckett 1989
8 STD; M	4 to 12 y	4 pregnant; followed for 1 y	RIA	1.287		10.0 - 45.3[8]	F-B[11] 1991
4 unspecified; unspecified	unspecified		nr	none	33.3		Hillyer 1992
12 various; 2S, 10M	6 to 19 y	sampled twice daily for 4 d	RIA	1.287	20.5	8.0 - 32.3[1]	Sojka 1993
12 various; 7G, 5M	2 to 16 y		RIA	none	21.4[5]	14.6 - 28.2[2,7]	Messer 1995a
6 various; 3G, 3M	4 to 16 y		RIA	none	19.9	11.5 - 28.2[2,3]	Messer 1995b
16 light breed; M	8 to 9 y	average levels over 25 d	RIA	1.287	23.2	19.1 - 27.2[2,3]	Sticker 1995

CF = factor used to convert from conventional units to Système International d'Unitès
*Single author or first of multiple authors, and date of publication

Appendix B. (continued)

STD = Standardbred horse, QH = Quarter horse, ARAB = Arabian horse
S = stallion, G = gelding, M = mare, both = male and female
d = day(s), mo = month(s), y = year(s)
CPB = competitive protein binding, RIA = radioimmunoassay, ELISA = enzyme-linked immunosorbent assay, nr = not reported
[1]Minimum and maximum values reported
[2]± 1.96 standard deviations from the mean
[3]Calculated from the reported standard error of the mean
[4]Values estimated from a graph
[5]Mean of two or more reported means
[6]Levels below the limit of detection of 5.0 nmol/L were assigned a value of 2.5 nmol/L for calculating the mean
[7]Mean of two or more reported standard deviations
[8]Highest and lowest reported means
[9]8:00, 12:00 and 16:00 hours
[10]20:00, 24:00 and 4:00 hours
[11]F-B = Flisińska-Bojanowska

Appendix C. Summary of reported triiodothyronine concentrations in the serum or plasma of horses from birth to 1 year

Number and breed	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
5 TB and STD	birth	umbilical cord blood	RIA	0.0154	8.1		Irvine 1975
5 unspecified	newborn	umbilical cord blood	RIA	none	8.1	4.0 - 12.2[1]	Irvine 1984
12 pony	birth	full term; umbilical cord blood	RIA	1.54	6.6	3.5 - 9.8[1,2]	Silver 1991
6 various	birth	1989 foals	RIA	0.0154	12.15	4.0 - 20.3[1]	Boosinger 1995
9 various	birth	1990 foals	RIA	0.0154	8.2	3.9 - 12.5[1]	Boosinger 1995
8 TB	< 1 hr	followed from birth to 28 d	RIA	none	12.8	-1.7 - 27.3[1]	Murray 1993
12 pony	2 hr	full term; peak neonatal levels	RIA	1.54	12.9	7.7 - 18.2[1,2]	Silver 1991
4 TB and STD	1 to 10 hr		RIA	0.0154	15.3		Irvine 1975
10 various	< 24 hr	5 TB-cross and 5 pony foals	RIA	0.0154	5.6	0.4 - 11.3[3]	Shaftoe 1988
3 TB and STD	1 to 3 d		RIA	0.0154	14.5		Irvine 1975

Appendix C. (continued)

Summary of reported triiodothyronine concentrations in the serum or plasma of horses from birth to 1 year

Number and breed	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
8 TB	2 d	followed from birth to 28 d	RIA	none	11.4	4.7 - 18.1[1]	Murray 1993
2 TB and STD	4 d		RIA	0.0154	14.4		Irvine 1975
8 TB	4 d	followed from birth to 28 d	RIA	none	7.8	-0.4 - 16.0[1]	Murray 1993
6 unspecified	4 to 6 d		RIA	none	14.4	1.1 - 27.8[1]	Irvine 1984
9 TB and STD	5 to 11 d		RIA	0.0154	9.7		Irvine 1975
8 TB	12 d	followed from birth to 28 d	RIA	none	5.6	3.2 - 8.0[1]	Murray 1993
5 TB and STD	12 to 16 d		RIA	0.0154	4.5		Irvine 1975
8 TB	20 d	followed from birth to 28 d	RIA	none	4.2	2.4 -6.0[1]	Murray 1993
8 TB	28 d	followed from birth to 28 d	RIA	none	3.1	2.3 - 5.6[1]	Murray 1993

Appendix C. (continued)

Summary of reported triiodothyronine concentrations in the serum or plasma of horses from birth to 1 year

Number and breed	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
5 TB and STD	22 to 90 d		RIA	0.0154	3.0		Irvine 1975
8 TB	foals		RIA	none	2.4	0.5 -3.7[3]	Blackmore 1978
14 TB and QH	1.5 to 4 mo		RIA	0.0154	3.0[4]	2.1 - 4.2[3]	Chen 1981
6 TB	6 mo	weaned, each sampled 4 times	RIA	0.0154		0.8 - 1.0[5]	Glade 1987
12 TB; unspecified	6 to 8 mo	weanlings	RIA	none	0.7[4,6]		Glade 1985

CF = factor used to convert from conventional units to Système International d'Unitès
*Single author or first of multiple authors, and date of publication
TB = Thoroughbred, STD = Standardbred horse, QH = Quarter horse
hr = hour(s), d = day(s), mo = month(s)
RIA = radioimmunoassay
[1]± 1.96 standard deviations from the mean

Appendix C. (continued)

2Standard deviation calculated from the reported standard error of the mean
[3]Minimum and maximum values reported
[4]Mean of two or more reported means
[5]Highest and lowest mean values reported
[6]Values estimated from a graph

Appendix D. Summary of reported thyroxine concentrations the serum or plasma of horses from birth to 1 year

Number and breed	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
8 TB and STD	birth	umbilical cord blood	CPB	12.87	494.7		Irvine 1975
8 unspecified	newborn	umbilical cord blood	RIA	none	557	345.3 - 768.7[1]	Irvine 1984
12 pony	birth	full term; umbilical cord blood	RIA	1.287	386.1	232.3 - 539.9[1,2]	Silver 1991
11 unspecified	newborn	5 minutes after birth	RIA	12.87	377.1		Dudan 1987
6 various	birth	1989 foals	RIA	12.87	576.6		Boosinger 1995
9 various	birth	1990 foals	RIA	12.87	357.8	221.6 - 494.0	Boosinger 1995
8 TB	< 1 hr	followed from birth to 28 d	RIA	none	492.9	378.8 - 607.0[1]	Murray 1993
12 pony	1 hr	full term; peak neonatal levels	RIA	1.287	396.4	227.7 -565.0[1,2]	Silver 1991
11 TB and STD	1 to 10 hr		CPB	12.87	371.4		Irvine 1975
10 various	< 24 hr	5 TB-cross and 5 pony	RIA	12.87	175.4	56.6 - 323.0[3]	Shaftoe 1988

Appendix D. (continued)

Summary of reported thyroxine concentrations in the serum or plasma of horses from birth to 1 year

Number and breed	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
6 TB and STD	1 to 3 d		CPB	12.87	360.7		Irvine 1975
11 unspecified	48 hr		RIA	12.87	263.8		Dudan 1987
8 TB	2 d	followed from birth to 28 d	RIA	none	357.8	252.2 - 463.4[1]	Murray 1993
6 TB and STD	4 d		CPB	12.87	143.6		Irvine 1975
8 TB	4 d	followed from birth to 28 d	RIA	none	231.7	110.6 - 352.8[1]	Murray 1993
6 unspecified	4 to 6 d		RIA	none	143	13.6 - 272.4[1]	Irvine 1984
15 TB and STD	5 to 11 d		CPB	12.87	95.8		Irvine 1975
8 TB	12 d	followed from birth to 28 d	RIA	none	60.0	5.5 - 114.5[1]	Murray 1993
4 TB and STD	12 to 16 d		CPB	12.87	35.0		Irvine 1984

Appendix D. (continued)

Summary of reported thyroxine concentrations in the serum or plasma of horses from birth to 1 year

Number and breed	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
8 TB	20 d	followed from birth to 28 d	RIA	none	36.7	18.3 - 55.1[1]	Murray 1993
8 TB	28 d	followed from birth to 28 d	RIA	none	30.6	-3.5 - 64.7[1]	Murray 1993
11 TB	unspecified	foals	RIA	none	48.7	11 - 123[3]	Blackmore 1978
5 STD	0 to 1 mo		CPB	12.87	57.8	29.8 - 85.8[1]	Lowe 1970
4 unspecified	0.5 to 2 mo		CPB	12.87	38.6	4.9 - 72.3[1]	Kallfelz 1973
6 TB and STD	22 to 90 d		CPB	12.87	33.1		Irvine 1975
14 TB and QH	1.5 to 4 mo		RIA	12.87	51.5[4]	37.3 - 67.6[3]	Chen 1981
16 STD	1 to 6 mo		CPB	12.87	33.5	9.8 - 57.2[2]	Lowe 1970
6 TB	6 mo	each sampled 4 times	RIA	1.287		29.6 - 34.7[5]	Glade 1987

Appendix D. (continued)

Summary of reported thyroxine concentrations in the serum or plasma of horses from birth to 1 year

Number and breed	Age	Additional comments	Assay	CF	Mean (nmol/L)	Range (nmol/L)	Reference*
8 TB	6 to 8 mo	weanlings	RIA	1.287	29.0[4]		Glade 1984
12 TB; unspecified	6 to 8 mo	weanlings	RIA	none	16.3[4,6]		Glade 1985
5 STD	6 to 12 mo		CPB	12.87	25.6	10.9 - 40.3[1]	Lowe 1970
10 crossbreds	yearlings	1969 yearlings	CPB	12.87	33.1	16.2 - 43.5[3]	Kallfelz 1970
11 crossbreds	yearlings	1970 yearlings	CPB	12.87	32.8	18.1 - 47.5[1]	Lowe 1970
4 unspecified	1 y		CPB	12.87	30.2	8.2 - 52.2[1]	Kallfelz 1973
47 TB	1 y		RIA	none	20.8[4]	0 - 39[3]	Blackmore 1978

CF = factor used to convert from conventional units to Système International d'Unités
*Single author or first of multiple authors, and date of publication

Appendix D. (continued)

TB = Thoroughbred, STD = Standardbred horse, QH = Quarter horse
hr = hour(s), d = day(s), mo = month(s), y = year(s)
CPB = competitive protein binding, RIA = radioimmunoassay
[1]± 1.96 standard deviations from the mean
[2]Standard deviation calculated from the reported standard error of the mean
[3]Minimum and maximum values reported
[4]Mean of two or more reported means
[5]Highest and lowest mean values reported
[6]Values estimated from a graph

Appendix E. Approximate time of selected events in the ossification of the thoracic limb of the horse and the status of sham-operated control foals

Bone	Event	Normal time of event	Foals	
			F18	F14
Scapula				
cranial glenoid cavity	appearance of ossification center	325 days to following birth	large	large
Humerus				
proximal epiphysis	appearance of ossification center	290 to 310 days	large	large
greater tubercle	appearance of ossification center	315 to 335 days	large	large
medial condyle	appearance of ossification center	315 to 335 days	large	large
distal epiphysis	appearance of ossification center	290 to 315 days	large	large
Ulna				
proximal epiphysis	appearance of ossification center	330 days to following birth	small	small
styloid process	appearance of ossification center	330 days to following birth	small	small
Radius				
proximal epiphysis	appearance of ossification center	315 to 335 days	large	large
distal epiphysis	appearance of ossification center	265 to 295 days	large	large

Appendix E. (continued)

Approximate time of selected events in the ossification of the thoracic limb of the horse and the status of sham-operated control foals

Bone	Event	Normal time of event	Foals	
			F18	F14
Third Metacarpal				
proximal epiphysis	appearance of ossification center	270 to 330 days	large	large
	closure of epiphysis	before birth	closed	closed
distal epiphysis	appearance of ossification center	265 to 290 days	large	large
Second and Fourth metacarpal				
proximal epiphysis	appearance of ossification center	late in gestation	large	large
Proximal sesamoids	appearance of ossification center	290 to 330 days	large	large
Proximal phalanx				
proximal epiphysis	appearance of ossification center	290 to 320 days	large	large
distal epiphysis	appearance of ossification center	265 to 310 days	large	large
	closure of epiphysis	before birth to 1 month of age	closed	closed

Appendix E. (continued)

Approximate time of selected events in the ossification of the thoracic limb of the horse and the status of sham-operated control foals

Bone	Event	Normal time of event	Foals	
			F18	F14
Middle phalanx				
proximal epiphysis	appearance of ossification center	290 to 335 days	large	large
distal epiphysis	appearance of ossification center	310 to 330 days	large	large
	closure of epiphysis	before birth to 1 week of age	closed	closed
Distal sesamoid	appearance of ossification center	325 to soon following birth	large	large
Distal phalanx				
proximal cap	appearance of ossification	late in gestation	large	large
	closure of epiphysis	before birth	closed	closed

days = days of gestation
small = < 50% of the cartilage anlage was ossified; large = > 50% of the cartilage anlage was ossified

Appendix F. Status of selected events in the ossification of the thoracic limb of partially thyroidectomized foals

Bone	Event	Foals			
		F10	F17	F19	F13
Scapula					
cranial glenoid cavity	appearance of ossification center	absent	absent	absent	absent
Humerus					
proximal epiphysis	appearance of ossification center	absent	small	large	large
greater tubercle	appearance of ossification center	absent	absent	small	small
medial condyle	appearance of ossification center	absent	absent	small	small
distal epiphysis	appearance of ossification center	absent	small	large	large
Ulna					
proximal epiphysis	appearance of ossification center	absent	absent	absent	absent
styloid process	appearance of ossification center	absent	absent	absent	absent
Radius					
proximal epiphysis	appearance of ossification center	absent	small	small	large
distal epiphysis	appearance of ossification center	small	small	large	large

Appendix F. (continued)

Status of selected events in the ossification of the thoracic limb of partially thyroidectomized foals

Bone	Event	Foals			
		F10	F17	F19	F13
Third metacarpal					
proximal epiphysis	appearance of ossification center	absent	absent	small	large
	closure of epiphysis	open	open	open	closed
distal epiphysis	appearance of ossification center	absent	large	large	large
Second and Fourth metacarpal					
proximal epiphysis	appearance of ossification center	absent	absent	absent	absent
Proximal sesamoids	appearance of ossification center	absent	absent	small	large
Proximal phalanx					
proximal epiphysis	appearance of ossification center	absent	small	large	large
distal epiphysis	appearance of ossification center	absent	small	large	large
	closure of epiphysis	open	open	open	open

Appendix F. (continued)

Status of selected events in the ossification of the thoracic limb of partially thyroidectomized foals

Bone	Event	Foals			
		F10	F17	F19	F13
Middle phalanx					
proximal epiphysis	appearance of ossification center	absent	small	large	large
distal epiphysis	appearance of ossification center	absent	absent	small	large
	closure of epiphysis	open	open	open	closed
Distal sesamoid	appearance of ossification center	absent	absent	absent	small
Distal phalanx					
proximal cap	appearance of ossification center	absent	absent	absent	large
	closure of epiphysis	open	open	open	closed

absent = no ossification of cartilage anlage
small = < 50% of cartilage anlage was ossified; large = > 50% of cartilage anlage was ossified.

Appendix G. Approximate time of selected events in the ossification of the pelvic limb of the horse and the status of sham-operated control foals

Bone	Event	Normal time of event	Foals	
			F18	F14
Femur				
proximal epiphysis	appearance of ossification center	230 to 300 days	large	large
greater trochanter	appearance of ossification center	230 to 300 days	large	large
distal epiphysis	appearance of ossification center	220 to 245 days	large	large
Patella	appearance of ossification center	325 days birth	small	small
Tibia				
proximal epiphysis	appearance of ossification center	265 to 300 days	large	large
tibial tuberosity	appearance of ossification center	290 to 320 days	large	large
distal epiphysis	appearance of ossification center	280 to 300 days	large	large
Tuber calcaneus				
epiphysis	appearance of ossification center	325 days to soon following birth	large	large

Appendix G. (continued)

Approximate time of selected events in the ossification of the pelvic limb of the horse and the status of sham-operated control foals

Bone	Event	Normal time of event	Foals	
			F18	F14
Third Metatarsal				
proximal epiphysis	appearance of ossification center	270 to 330 days	large	large
	closure of epiphysis	before birth	closed	closed
distal epiphysis	appearance of ossification center	265 to 290 days	large	large
Second and Fourth metatarsal				
proximal epiphysis	appearance of ossification center	late in gestation	large	large
Proximal sesamoids	appearance of ossification center	290 to 330 days	large	large
Proximal phalanx				
proximal epiphysis	appearance of ossification center	290 to 320 days	large	large
distal epiphysis	appearance of ossification center	265 to 310 days	large	large
	closure of epiphysis	before birth to 1 month of age	closed	closed

Appendix G. (continued)

Approximate time of selected events in the ossification of the pelvic limb of the horse and the status of sham-operated control foals

Bone	Event	Normal time of event	Foals	
			F18	F14
Middle phalanx				
proximal epiphysis	appearance of ossification center	290 to 335 days	large	large
distal epiphysis	appearance of ossification center	310 to 330 days	large	large
	closure of epiphysis	before birth to 1 week of age	closed	closed
Distal sesamoid	appearance of ossification center	325 days to soon following birth	large	large
Distal phalanx				
proximal cap	appearance of ossification	late in gestation	large	large
	closure of epiphysis	before birth	closed	closed

days = days of gestation
small = < 50% of the cartilage anlage was ossified; large = > 50% of the cartilage anlage was ossified.

Appendix H. Status of selected events in ossification of the pelvic limb of partially thyroidectomized foals

Bone	Event	Foals			
		F10	F17	F19	F13
Femur					
proximal epiphysis	appearance of ossification center	absent	small	small	large
greater trochanter	appearance of ossification center	absent	small	small	large
distal epiphysis	appearance of ossification center	small	large	large	large
Patella	appearance of ossification center	absent	absent	absent	small
Tibia					
proximal epiphysis	appearance of ossification center	absent	large	large	large
tibial tuberosity	appearance of ossification center	absent	absent	small	large
distal epiphysis	appearance of ossification center	absent	small	large	large
Tuber calcaneus					
epiphysis	appearance of ossification center	absent	absent	absent	small
Third Metatarsal					
proximal epiphysis	appearance of ossification center	absent	absent	absent	large
	closure of epiphysis	open	open	open	open
distal epiphysis	appearance of ossification center	small	large	large	large

Appendix H. (continued)

Status of selected events in ossification of the pelvic limb of partially thyroidectomized foals.

Bone	Event	Foals			
		F10	F17	F19	F13
Second and Fourth metatarsal					
proximal epiphysis	appearance of ossification center	absent	absent	absent	absent
Proximal sesamoids	appearance of ossification center	absent	absent	small	large
Proximal phalanx					
proximal epiphysis	appearance of ossification center	absent	small	small	large
distal epiphysis	appearance of ossification center	absent	large	large	large
	closure of epiphysis	open	open	open	open
Middle phalanx					
proximal epiphysis	appearance of ossification center	absent	small	large	large
distal epiphysis	appearance of ossification center	absent	absent	small	large
	closure of epiphysis	open	open	open	closed
Distal sesamoid	appearance of ossification center	absent	absent	absent	small

Appendix H. (continued)

Status of selected events in ossification of the pelvic limb of partially thyroidectomized foals.

Bone	Event	Foals			
		F10	F17	F19	F13
Distal phalanx					
proximal cap	appearance of ossification	absent	absent	absent	large
	closure of epiphysis	open	open	open	open

absent = no ossification of the cartilage anlage
small = < 50% of the cartilage anlage was ossified; large = > 50% of cartilage anlage was ossified

Appendix I. Summary of the independent variables investigated for association with the syndrome of thyroid gland hyperplasia and musculoskeletal deformities in foals

Abridged description of variables: possible responses
Animal signalment
Breed of dam: Arab/mixed/other/quarter horse/standardbred/Thoroughbred
Age of dam: number of years
Number of pregnancies of this dam, including 1992-93: number > 0
Dam has competed or dam was a standardbred or Thoroughbred and has raced: no/yes
Breed of foal: Arab/mixed/other/quarter horse/standardbred/Thoroughbred
Sex of foal: female/male
Length of gestation: number of days

Appendix I. (continued)

Summary of the independent variables investigated for association with the syndrome of thyroid gland hyperplasia and musculoskeletal deformities in foals

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Abridged description of variables: possible responses

Pasture

Dam kept on native grass/improved (reseeded) pasture during summer: no/yes

Pastures irrigated/fertilized/treated with pesticide/treated with herbicide in 1992: no/yes

Diet

Dam regularly fed hay/greenfeed/silage/other forage - during summer/during winter: no/yes

Dam regularly fed grain (oats, barley, wheat, other)/complete horse feed - during summer/during winter: no/yes

Dam regularly fed protein/protein-vitamin-mineral supplement - during summer/during winter: no/yes

Dam regularly had access to salt or mineral blocks/access to loose salt or mineral/salt or mineral added to concentrate - during summer/during winter: no/yes

Appendix I. (continued)

Summary of the independent variables investigated for association with the syndrome of thyroid gland hyperplasia and musculoskeletal deformities in foals

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Abridged description of variables: possible responses

Diet (continued)

Dam regularly had access to water supplied to local city/from local well/from dug-out/from stream or river/from other source
- during summer/during winter: no/yes

Farm

Number of years owners/operators have been producing foals at current location: number > 0

Dam regularly had access to trees and/or bushes: no/yes

Dam regularly exposed to fences and/or buildings treated with paint or stain/creosote/diesel fuel/used motor oil: no/yes

Number of horses that came onto farm during 1992: 0/1 or 2/3 to 10/more than 10

Cattle/other livestock (pigs, sheep, poultry) kept on the farm in 1992: no/yes

Appendix I. (continued)

Summary of the independent variables investigated for association with the syndrome of thyroid gland hyperplasia and musculoskeletal deformities in foals

Abridged description of variables: possible responses
Dam management
Dam received ivermectin in paste for horses/ivermectin injectable for cattle given orally/other anthelmintic prior to foaling: no/yes
Dam was vaccinated against rabies/equine encephalitis/equine influenza/equine rhinopneumonitis/equine viral arteritis/tetanus/strangles/Potomac horse fever prior to foaling: no/yes
Dam was artificially inseminated/bred naturally during her last estrus period in 1992: no/yes
Dam received a drug or medication prior to foaling: no/yes
Dam was off or new to the farm during gestation: no/yes