THE UNIVERSITY OF MANITOBA

STUDIES ON OVINE PLACENTAL LACTOGEN

by

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DOCTOR OF PHILOSOPHY

To

My parents and wife:

Mr. & Mrs. Kai-On Chan

and

Mrs. Ting-Mei Chan

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LIST OF ABBREVIATION

Peptide Hormones:

ACTH	Adrenocorticotropic hormone
CG	Chorionic Gonadotropin
FSH	Follicle Stimulating Hormone
GH	Growth Hormone
LH	Luteinizing Hormone
PRL	Prolactin
PL	Placental Lactogen

Prefix to hormones:

b	bovine
h	human
o	ovine
r	rat
c	caprine
mou	mouse
m	monkey
gp	guinea pig
ham and p	hamster and porcine

Others:

C	Degree Centigrade
cpm	Counts per minute
g	Gram
mg	Milligram
ug	Microgram

M	Molar
Mm	Millimolar
ul	Microliter
N	Normal
ng	Nanogram
l	Liter
g	Gravitational force
IU	International unit
U	Unit

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ABSTRACT

STUDIES ON OVINE PLACENTAL LACTOGEN (oPL). John S.D. Chan, Dept. of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada

Using conventional protein purification procedures and radioreceptor assay for growth hormone (RRA-GH), oPL was purified to near homogeneity (greater than 2,000-fold) from ovine placental cotyledons. The molecular weight of oPL is approximately 21,000 as determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Specific binding sites for oPL have been found in ovine liver (maternal and fetal), adipose tissue, ovary, corpus luteum, and non-pregnant uterus. Specific studies showed that only growth hormone preparations could displace oPL binding to its receptors whereas other hormone preparations could not, indicating that oPL binds specifically to GH-receptor sites in ovine tissues. By employing the radioreceptor assay for placental lactogen (PL) using oPL and ovine liver as hormone standard and receptor respectively, PL-like activity was detected in placental extracts of human, monkey, goat, guinea pig, and mouse but not in cow, pig, horse, dog, rat, and rabbit. Homologous radioimmunoassay (RIA) for oPL has been developed in which ovine pituitary prolactin (oPRL) and growth hormone (oGH) as well as other pituitary and placental hormones from several species exhibit no cross-reaction. Using this RIA, oPL is detectable in the uterine vein blood samples as early as 26 days of gestation.

The secretory pattern of oPL in the maternal circulation is similar to that of human and monkey placental lactogen with the peak level in both circulation and placentomes after 80 days of gestation. oPL is found in the fetal circulation and allantoic fluids throughout pregnancy with high levels during early pregnancy. oPL is not detectable in amniotic fluid and maternal urine. oPL is dectable in extracts of chorionic membranes and maternal caruncles as early as 16 days of gestation with high concentration in fetal membranes. These studies indicate that oPL is secreted from fetal tissues. This is supported by the biosynthesis studies which showed that oPL is synthesized and secreted by chorio-allantoic membranes as early as 22 days of pregnancy. The half-time disappearance rate ($t_{\frac{1}{2}}$) of oPL in sheep is approximately 60 min. When oPRL and oGH were measured by specific RIAs in maternal blood samples during pregnancy, the levels of oPRL are inversely related to those of oPL without any major variation in oGH levels. This finding suggests that oPL might be the hormone that suppresses oPRL secretion during pregnancy. In the bioassay using hypophysectomized rat tibial width increase as an index of somatogenic effects, oPL is 1.5 times more potent than a bovine GH (1.0 U/mg) standard. In pseudopregnant rats, administration of oPL into the animals can prevent the loss of LH-receptors in the corpora lutea and the fall of progesterone after PGF_{2a} or bromoergocryptine (CB-154) injection. These studies suggest that during pregnancy oPL may act as a "growth hormone" of pregnancy and that it helps to maintain the integrity of LH-receptors in the corpora lutea. Finally,

receptor-binding studies show that only oPL and human growth hormone (hGH) bind to animal and human tissue receptors whereas other growth hormone preparations from other species do not. These studies suggest that the binding sites in oPL and hGH are very similar in conformation. Thus, further structural analysis on the active sites for binding and for growth promoting activity may have potential implication for future clinical use.

SECTION 1 REVIEW OF PLACENTAL PROTEIN AND POLYPEPTIDE HORMONES

A. INTRODUCTION:

The importance of the placenta as an active participant in providing for an intrauterine milieu favorable for fetal survival has been surmised since antiquity. Modern investigative techniques have, however, removed this subject from the area of conjecture and provided understanding of the mechanisms by which placental tissue carries out some of its functions. One of the best known capabilities of this highly developed, though transitory, tissue is the maintenance of fetal-maternal gradients in terms of gases and metabolites which are favorable to the fetus. Less well understood, however, is the role played by the placenta as an endocrine organ. Study of the secretions of placental tissue has been hindered by several problems which were not encountered in the classical studies of the pituitary and its target organs. The method of total extirpation and replacement treatment, for example, is denied the investigator of placental endocrinology. Furthermore, as our knowledge of the endocrine functions of the placenta has grown it is becoming apparent that, unlike the secretions of the anterior pituitary, the secretions of the placenta vary widely from one species to another. Variation is also apparent within a given species at different stages of gestation. Such problems were not to become a prohibitive barrier to investigation, however, and recent