

09PH  
T164



INVESTIGATION OF GALACTOSYLTRANSFERASE  
AND  $\alpha$ -LACTALBUMIN-LIKE PROTEINS IN  
MAMMALIAN REPRODUCTIVE TRACTS

by

Yulu Tang, M. Sc. Agri.

A thesis submitted to the University of Adelaide  
in fulfilment of the requirement for the degree of Doctor of Philosophy

Department of Animal Sciences  
Waite Agricultural Research Institute  
The University of Adelaide

October, 1993

A 11 4 1994

# TABLE OF CONTENTS

TABLE OF CONTENTS	i
ABSTRACT	x
DECLARATION	xii
ACKNOWLEDGMENTS	xiii
PUBLICATIONS	xv
ABBREVIATIONS	xvi
<b>GENERAL INTRODUCTION</b>	<b>1</b>
<b>CHAPTER 1      LITERATURE REVIEW</b>	<b>3</b>
<b>1.1            SOME SECTIONS OF MAMMALIAN                   REPRODUCTIVE TRACTS</b>	<b>3</b>
1.1.1          Epididymis, Epididymal Fluid and Epididymal Sperm	3
1.1.1.1        Epididymis	3
1.1.1.2        Epididymal Fluid	3
1.1.1.3        Major Epididymal Secretory Proteins in the Rat	4
1.1.1.4        Sperm Maturation in the Epididymis	6
1.1.2          Uterus and Uterine Fluid	6
1.1.2.1        Uterus	6
1.1.2.2        Uterine Fluid	7
<b>1.2            EVENTS AND CHARACTERISTICS OF                   FERTILIZATION IN MAMMALS</b>	<b>8</b>
1.2.1          Structures of Mammalian Spermatozoon and Egg	8

1.2.1.1	Structure of Mammalian Spermatozoon	8
1.2.1.2	Structures of Mammalian Egg	10
1.2.2	General Events of the Process of Mammalian Fertilization	10
1.2.2.1	Sperm Capacitation	11
1.2.2.2	Sperm Acrosome Reaction	11
1.2.2.3	Sperm Penetration through the Cumulus Oophorus	12
1.2.2.4	Sperm Binding to Zona Pellucida	13
1.2.2.5	Sperm Penetration through Zona Pellucida	15
1.2.2.6	Sperm-Egg Fusion	16
1.2.3	Molecular Mechanism in Mammalian Sperm-Zona Binding	16
1.2.3.1	Receptors of Zona Pellucida for Sperm	16
1.2.3.2	Receptors of Sperm for Zona Pellucida	18
1.2.3.2.1	Saccharides Possibly Involved in Sperm-Zona Pellucida Binding	18
1.2.3.2.2	Lectin-Like Proteins	19
1.2.3.2.3	Enzymes	21
1.2.3.2.4	Signal Transduction Proteins	23
1.2.3.2.5	Other Proteins	24
1.2.4	Species Specificity	25
<b>1.3</b>	<b>GALACTOSYLTRANSFERASE AND <math>\alpha</math>-LACTALBUMIN</b>	<b>27</b>
1.3.1	General Introduction	27
1.3.1.1	Enzyme Reaction and Terminology of Galactosyl- transferase	27
1.3.1.2	Relationship between Galactosyltransferase and $\alpha$ -Lactalbumin	28
1.3.2	Distribution of Galactosyltransferase and $\alpha$ -Lactalbumin	29
1.3.3	Enzymatic Properties of Galactosyltransferase	30
1.3.3.1	Regulation of $\alpha$ -Lactalbumin on Galactosyltransferase	

	Reaction	30
1.3.3.2	Metal Ion Requirement of Galactosyltransferase	31
1.3.3.3	Acceptor Specificity of Galactosyltransferase	32
1.3.3.4	Inhibition of Galactosyltransferase Activity	33
1.3.3.5	Interaction of Galactosyltransferase with $\alpha$ -Lactalbumin, Mn <sup>2+</sup> , UDP-Galactose and Acceptors	34
1.3.3.6	K <sub>M</sub> Values of Galactosyltransferase	35
1.3.4	Chemical Aspects of Galactosyltransferase and $\alpha$ -Lactalbumin	35
1.3.5	Biological Functions of Galactosyltransferase	36
1.3.6	Some Methods Used in Studies of Galactosyltransferase and $\alpha$ -Lactalbumin	37
1.3.6.1	Purification and Stabilization of Galactosyltransferase	37
1.3.6.2	Assay Methods for Galactosyltransferase Activity and $\alpha$ -Lactalbumin Activity	38
1.3.6.2.1	Assay Methods	38
1.3.6.2.2	Some Factors Affecting the Assays	39
<b>CHAPTER 2</b>	<b>GENERAL MATERIALS AND METHODS</b>	<b>41</b>
<b>2.1</b>	<b>MATERIALS</b>	<b>41</b>
<b>2.2</b>	<b>METHODS</b>	<b>41</b>
2.2.1	Galactosyltransferase Assay and $\alpha$ -Lactalbumin Assay	41
2.2.2	Assay of Protein Concentration	42

<b>CHAPTER 3</b>	<b>GALACTOSYLTRANSFERASE ACTIVITY</b>	
	<b>IN EPIDIDYMAL PLASMA AND RETE TESTIS PLASMA</b>	
	<b>OF SOME MAMMALS</b>	<b>44</b>
<b>3.1</b>	<b>INTRODUCTION</b>	<b>44</b>
<b>3.2</b>	<b>MATERIALS AND METHODS</b>	<b>45</b>
3.2.1	Literature Review of the Method	45
3.2.2	Chemicals	45
3.2.3	Animals	46
3.2.4	Methods	46
3.2.4.1	Sample Collection	46
3.2.4.2	Assay of Galactosyltransferase Activity and Pyrophosphatase and Phosphatase Activities	47
3.2.4.3	Assay of Protein Concentration	48
<b>3.3</b>	<b>RESULTS</b>	<b>48</b>
3.3.1	Assay Method	48
3.3.1.1	Pyrophosphatase and Phosphatase Activities in Cauda Epididymal Plasma	48
3.3.1.2	Assay Conditions for Comparison of Galactosyl- transferase Activities in Various Species	50
3.3.2	Comparison of Galactosyltransferase Activities (together with Comparison of Pyrophosphatase and Phosphatase Activities) in Cauda Epididymal Plasma of Various Species	51
3.3.3	Comparison of Cauda Epididymal Plasma Galactosyl- transferase and Serum Galactosyltransferase	52
3.3.4	Galactosyltransferase Activity in Rete Testis Plasma	52
<b>3.4</b>	<b>DISCUSSION</b>	<b>60</b>

<b>CHAPTER 4 SOME STUDIES ABOUT GALACTOSYL- TRANSFERASE AND MAMMALIAN SPERM-ZONA PELLUCIDA BINDING</b>	<b>64</b>
<b>GENERAL INTRODUCTION OF CHAPTER 4</b>	<b>64</b>
<b>PART 1 (4-1) EXAMINATION OF GALACTOSYL- TRANSFERASE ACTIVITY IN SPERM PREPARATIONS OF THE RAT AND RAM</b>	<b>73</b>
<b>4-1.1 INTRODUCTION</b>	<b>73</b>
<b>4-1.2 MATERIALS AND METHODS</b>	<b>73</b>
4-1.2.1 Animals	73
4-1.2.2 Methods	74
4-1.2.2.1 Examination of Sperm Surface Galactosyltransferase Activity	74
4-1.2.2.1.1 Sperm Preparation	74
4-1.2.2.1.2 Assay of Sperm Surface Galactosyltransferase Activity	75
4-1.2.2.2 Assessment of Acrosome Status	76
4-1.2.2.3 Purification of Mouse Sperm by Percoll Gradient Centrifugation	77
<b>4-1.3 RESULTS</b>	<b>78</b>
4-1.3.1 Galactosyltransferase Activity in Sperm Preparation	78
4-1.3.2 Acrosome Status of Rat and Ram Sperm during the Procedure for Sperm Surface Galactosyltransferase Assay	79
<b>4-1.4 DISCUSSION</b>	<b>83</b>
<b>PART 2 (4-2) BINDINGS OF RAT AND RAM SPERM TO MOUSE ZONA PELLUCIDA</b>	<b>87</b>
<b>4-2.1 INTRODUCTION</b>	<b>87</b>

<b>4-2.2</b>	<b>LITERATURE REVIEW OF MAMMALIAN <i>IN VITRO</i> FERTILIZATION (IVF)</b>	88
4-2.2.1	Parameters of IVF Methodology	88
4-2.2.2	<i>In Vitro</i> Fertilization in the Mouse, Rat and Sheep	91
<b>4-2.3</b>	<b>MATERIALS AND METHODS</b>	95
4-2.3.1	Materials	95
4-2.3.2	Preparation of Culture Medium	95
4-2.3.3	Preparation of Mouse Eggs	98
4-2.3.4	Preparation of Sperm	98
4-2.3.5	Sperm-Zona Pellucida Binding Assay	100
<b>4-2.4</b>	<b>RESULTS</b>	101
4-2.4.1	Binding of Mouse, Rat and Ram Sperm to Mouse Zona	101
4-2.4.2	Effect of Sperm Concentration on the Binding of Mouse, Rat and Ram Sperm to Mouse Zona	101
4-2.4.3	Effect of Incubation Time on the Binding of Mouse, Rat and Ram Sperm to Mouse Zona	102
4-2.4.4	Binding Affinities of Mouse, Rat and Ram Sperm to Mouse Zona	102
<b>4-2.5</b>	<b>DISCUSSION</b>	110
<b>PART 3 (4-3)</b>	<b>EFFECTS OF UDP-GALACTOSE ON THE BINDING OF RAT SPERM AND RAM SPERM TO MOUSE ZONA PELLUCIDA</b>	113
<b>4-3.1</b>	<b>INTRODUCTION</b>	113
<b>4-3.2</b>	<b>MATERIALS AND METHODS</b>	113
4-3.2.1	Animals	113
4-3.2.2	Assay of the Effect of UDP-galactose on Sperm-Zona Binding <i>In Vitro</i>	113
<b>4-3.3</b>	<b>RESULTS</b>	114

4-3.3.1	Inhibition of UDP-galactose on Mouse Sperm-Zona Binding	114
4-3.3.2	Effects of UDP-galactose on the Binding of Mouse, Rat and Ram Sperm to Mouse Eggs	115
4-3.4	<b>DISCUSSION</b>	116
	<b>GENERAL DISCUSSION OF CHAPTER 4</b>	118
	<b>CHAPTER 5 GALACTOSYLTRANSFERASE IN FEMALE REPRODUCTIVE TRACT FLUID</b>	
	<b>--- DETECTION AND KINETIC COMPARISON WITH SERUM GALACTOSYLTRANSFERASE</b>	120
5.1	<b>INTRODUCTION</b>	120
5.2	<b>MATERIALS AND METHODS</b>	120
5.2.1	Preparation of Uterine Flushings	120
5.2.2	Preparation of Ewe Serum	121
5.2.3	Galactosyltransferase Activity Assay	122
5.3	<b>RESULTS</b>	122
5.3.1	Reactions of Galactosyltransferase in Ewe Uterine Flushings to $\alpha$ -Lactalbumin	122
5.3.2	Effect of UDP-galactose Concentration	122
5.3.3	Effect of <i>N</i> -acetylglucosamine Concentration	123
5.3.4	Effect of $Mn^{2+}$ Concentration	123
5.3.5	Effect of Varying Divalent Cations	123
5.3.6	Effect of pH	124
5.3.7	Effect of Incubation Temperature	124
5.3.8	Comparison of the Galactosyltransferase Activities	



	Normalized to Protein Concentration in the Uterine	
	Flushings and in the Serum of Ewes	124
5.3.9	Effects of Protein Concentration and Incubation Time	124
<b>5.4</b>	<b>DISCUSSION</b>	<b>138</b>
<b>CHAPTER 6</b>	<b>INVESTIGATIONS OF <math>\alpha</math>-LACTALBUMIN- LIKE ACTIVITY IN MAMMALIAN EPIDIDYMIS</b>	<b>140</b>
<b>6.1</b>	<b>INTRODUCTION</b>	<b>140</b>
<b>6.2</b>	<b>MATERIALS AND METHODS</b>	<b>145</b>
6.2.1	Animals	145
6.2.2	Chemicals	145
6.2.3	Methods	145
6.2.3.1	Preparation of a Low $M_r$ Protein Fraction of Rat Epididymal Extract	145
6.2.3.1.1	Collection of Rat Epididymides	145
6.2.3.1.2	Preparation of a Low $M_r$ Protein Fraction by Sephadex G-75 Chromatography	146
6.2.3.1.3	Elimination of the Remaining GalTase Activity in the Low $M_r$ Protein Fraction	146
6.2.3.2	Preparation of Epididymal Plasma from Various Mammalian Species	147
6.2.3.3	Preparation of a Protein Fraction of Rat Epididymal Extract by Ammonium Sulphate Precipitation	147
6.2.3.4	$\alpha$ -Lactalbumin-Like Activity Assay	148
6.2.3.5	High-Voltage Paper Electrophoresis Analysis of the Product(s) in the $\alpha$ -Lactalbumin-Like Activity Assays of the Low $M_r$ Protein Fraction	149

6.2.3.6	Galactinol Standard	150
6.2.3.7	Gas Chromatography Examination of Degradation Product of UDP-galactose by Rat Epididymal Preparation	151
6.2.3.8	SDS-Polyacrylamide Gel Electrophoresis and Gel Staining	152
6.2.3.9	Assay of Protein Concentration	152
<b>6.3</b>	<b>RESULTS</b>	152
6.3.1	Investigation of $\alpha$ -Lactalbumin-Like Activity in Epididymal Plasma of Different Mammals	152
6.3.2	Investigation of $\alpha$ -Lactalbumin-Like Activity in the Low $M_r$ Protein Fraction of Sephadex G-75 Chromatography	153
6.3.2.1	The Low $M_r$ Protein Fraction from Sephadex G-75 Chromatography	153
6.3.2.2	Assay of $\alpha$ -lac-Like Activity in the Low $M_r$ Protein Fraction	154
6.3.2.3	High-Voltage Paper Electrophoresis Analysis of the Product(s) in the $\alpha$ -Lactalbumin-like Activity Assays of the Low $M_r$ Protein Fraction	155
6.3.2.4	Gas Chromatography Examination of Degradation Product of UDP-galactose by Rat Epididymal Preparation	155
6.3.3	Stimulation of Galactosyltransferase Activity by Bovine Serum Albumin	156
6.3.4	Investigation of $\alpha$ -Lactalbumin-like Activity in the Protein Fraction of Rat Epididymal Extract Precipitated by 40-60% Saturated Ammonium Sulphate	157
<b>6.4</b>	<b>DISCUSSION</b>	166
<b>CHAPTER 7</b>	<b>GENERAL DISCUSSION</b>	171
<b>BIBLIOGRAPHY</b>		174

## ABSTRACT

The studies undertaken in this thesis concern the functions of galactosyltransferase (GalTase) and  $\alpha$ -lactalbumin ( $\alpha$ -lac) in mammalian reproduction. The following results have been obtained.

(a) GalTase activity is commonly present in the epididymal plasma of various mammalian species examined including mouse, rat, rabbit, ram and boar. Under the assay conditions which inhibited pyrophosphatase and alkaline phosphatase activities, the levels of the activities in different species were similar. However, it was found that the pyrophosphatase and alkaline phosphatase activities, which inhibit GalTase activity, of epididymal plasma differed very much between these species. This suggests that under physiological conditions, the GalTase activity of epididymal plasma is inhibited to very different degrees between these species. Therefore, GalTase may not have an important function at least in the epididymal plasma of the species with high pyrophosphatase and alkaline phosphatase activities in the plasma.

(b) Soluble GalTase activity is present in the ewe uterine lumen. Comparisons of the kinetic parameters of the uterine luminal enzyme and those of the serum enzyme of ewe suggest that the luminal enzyme is not transported from blood. This study is an initial investigation of soluble GalTase in the lumen of female reproductive tract. When further investigations are carried out, the comparisons between the enzyme status in oviduct lumen and that in uterine lumen in different species should yield important data concerning the function of GalTase in sperm-zona pellucida binding.

(c) Both rat sperm and ram sperm can bind to mouse zona pellucida. GalTase activity has been detected only in the sperm preparation of ram but not rat using the current method. UDP-galactose does not show specific inhibition on the binding of either rat or ram sperm to mouse zona. These results do not suggest that GalTase mediates the sperm-zona binding in rat and ram.

(d) There is no  $\alpha$ -lac-like activity detectable in epididymal extracts of various mammals including rat. Two essential problems have been found to cause experimental artefacts in the previous reports about  $\alpha$ -lac-like proteins. These results invalidate the suggestions from some of the previous reports about the special functions of GalTase in epididymal plasma under the regulation of  $\alpha$ -lac-like proteins, and suggest that the epididymal plasma GalTase may just catalyze the galactosylation of glycoproteins or glycolipids as the enzyme<sup>does</sup> elsewhere (not including those in milk) in the body.