

# 计划生育生殖生物学国家重点实验室 简况和论文选集

State Key Laboratory of Reproductive Biology  
Brief Introduction and Selected Papers

1997-1998

中国科学院动物研究所

Institute of Zoology, Chinese Academy of Sciences

北京 中国

Beijing China

## 实验室简介:

实验室主任: 祝 诚 研究员  
 副主任: 段恩奎 研究员  
 朴允尚 研究员  
 谢汝忠 高级实验师  
 学术委员会主任: 庄临之 研究员  
 副主任: 刘以训 研究员  
 李伟雄 研究员

学术委员会委员 (按姓氏笔画)

姓 名	性 别	专 业	职 称	单 位
王永潮	男	细胞生物学	教 授	北京师范大学生物系
王寒正	女	生殖生物学	研究员	上海计生所
石其贤	男	生殖生物学	研究员	浙江医科院计划生育所
庄临之	女	生殖生物学	研究员	中科院动物研究所
刘以训	男	生殖生物学	研究员	中科院动物研究所
孙曼雾	男	分子药理学	研究员	军事医学科学院药理毒理研究所
(科学院院士)				
李伟雄	男	生殖内分泌	研究员	国家计生委科技研究所
肖碧莲	女	生殖内分泌	研究员	国家计生委科技研究所
(工程院院士)				
严缘昌	男	细胞生物学	研究员	中科院上海细胞所
陈大元	男	生殖生物学	研究员	中科院动物研究所
祝 诚	男	生殖生物学	研究员	中科院动物研究所
顾之萍	女	生殖药理学	研究员	中科院上海药物研究所
龚岳亭	男	生物化学	研究员	中科院上海生化所
(科学院院士)				
曹咏清	女	生殖生物学	研究员	中科院动物研究所
薛社哲	男	细胞生物学	研究员	中国医学科学院基础医学研究所
(科学院院士)				

## 学术委员会顾问名单

姓 名	专 业	职 称	单 位
赵向前	生殖生物学	研究员	国家计生委科技司司长
朱耀华	计划生育	副研究员	国家计生委科技司
程治平	生殖生理学	教 授	哈尔滨医科大学
李载平	生物化学	研究员	中科院上海生物化学研究所
王 宾	分子遗传学	副教授	美国宾州大学医学院
Fuett, D. J.	生殖生物学	教 授	美国佐治亚大学生化系主任
Futhalla, M. F.	生殖生物学	教 授	美国人口委员会杰出专家
Segal, S. J.	生殖生物学	教 授	美国洛氏基金会人口科学部顾问
Luong, C. K. P.	生殖生物学	教 授	加拿大不列颠哥伦比亚大学妇产科系
Tsang, K. B.	生殖生物学	教 授	加拿大渥太华大学妇产科系 生殖生物中心主任

## 实验室研究方向:

1995 年经学术委员会讨论, 计划生育生殖生物学国家重点实验室的研究方向已集中为三大方面: 1) 配子发生、成熟、排放以及黄体形成、萎缩的分子机理; 2) 受精的分子机理和生殖工程; 3) 胚胎着床的分子机理和抗着床作用。目的是建立高质量的基础研究, 从形态学、生理学、生物化学、细胞生物学、分子生物学不同角度, 在细胞和分子水平上探讨生殖调控的基本规律, 同时为发展有效、安全、经济并容易被使用者掌握的避孕方法奠定基础。

## 研究组的构成及研究内容:

本实验室共设七个研究组, 在学科和技术方面各有特长, 从不同角度和不同水平探讨生殖规律。

1. 性腺生物学研究组: 灵长类排卵、受精和黄体溶解过程中 PA-PAI 基因表达的旁分泌和自分泌调节, 灵长类胎盘形成。  
负责人: 刘以训 研究员
2. 受精生物学研究组: 受精机制、显微授精与动物克隆。  
负责人: 孙曹原 研究员 博士
3. 胚胎生物学研究组: 胚胎植入启动的分子机理研究。  
负责人: 段思奎 研究员 博士

4. 生殖生理研究组：金属蛋白酶、PA 和生长因子在排卵、着床和黄体分泌中的作用。  
负责人：祝 诚 研究员
5. 生殖生物化学研究组：DNA 疫苗抗生育以及着床相关蛋白质在胚胎着床和生殖免疫中的作用研究。  
负责人：彭景丰 副研究员 博士
6. 生殖内分泌研究组：神经肽和神经递质在人胎盘和生殖轴的分离、定位及其功能的研究。  
负责人：王 红 研究员
7. 细胞和分子生物学实验室：人胎盘和子宫细胞增殖分化和激素分泌作用的调节；着床过程中基因调节和信号传导的研究。  
负责人：朴允尚 研究员 博士

## 课题申请指南：

所有课题申请将围绕计划生育生殖生物学国家重点实验室的三大研究方向进行、

1. 生殖内分泌学研究
2. 生殖腺的细胞和分子生物学研究
3. 生殖细胞发育、成熟和排放机理的研究
4. 受精机理和生殖工程研究
5. 胚胎着床分子机理的研究
6. 妊娠早期人胎盘的细胞和分子生物学研究
7. 与生殖相关的生物活性物质（蛋白质、细胞因子、激素）的基因调控与基因工程的研究
6. 有关避孕药物作用机制、开发应用与产业化的研究

# 1997 年资助课题一览表

序号	课题名称	申请人	起始年限	当年资助(万)
1	猴精液中纤溶酶原激活因子来源与功能的研究	邹如金	96.6-97.12	1.8
2	蜕膜免疫调节活性及其初步纯化的研究	颜建华	96.6-98.5	2.0
3	对 Le <sup>x</sup> 寡糖特异的单抗(AH <sub>6</sub> )阻断小鼠胚泡着床的机理研究	朱正美	96.6-98.5	1.8
4	着床相关糖蛋白生物化学及免疫学特性的研究	宋济范	96.6-98.5	1.8
5	人子宫内膜蜕膜中血管紧张素及其受体的研究	王 红	96.6-98.5	2.0
6	非整合素类层粘连蛋白受体在滋养层侵入的作用及其基因表达	段恩奎	96.6-98.5	2.0
7	人卵泡闭锁调控机制的研究	陈士岭	97.5-99.5	2.0
8	泛素系统降解蛋白质在小鼠精子细胞变态过程中的作用	王永潮	97.9-99.8	2.0
9	着床过程蜕膜细胞 CAM 的研究	李 宾	97.5-98.5	2.0
10	人细胞滋养层中金属蛋白酶表达调节机制的研究	朴允尚	97.5-99.4	2.0
11	应用反义技术研究细胞因子对胚泡植入的作用机制	曾灵芳	97.5-99.4	2.0
12	受精的细胞和分子生物学研究	陈大元	97.4-98.4	5.2
13	性腺的细胞和分子生物学研究	刘以训	97.4-98.4	5.2
14	人滋养层细胞植入过程与蜕膜间相互调节的细胞和分子机理	庄临之	97.4-98.4	1.5
15	细胞因子对胚泡着床过程中胚泡发育和子宫内膜的影响	王 妮	97.4-98.4	4.5
16	金属蛋白酶及其抑制剂在哺乳动物着床过程中的作用及基因表达	祝 诚	97.4-98.4	4.6
17	细胞外基质在小鼠胚泡着床中的作用	段恩奎	97.4-98.4	4.0
18	人早期胎盘的神经内分泌学研究	张崇理	97.4-98.4	1.2

## 1998 年国家重点实验室资助课题一览表

### 97 年延续课题:

序号	课题名称	申请人	起始年限	当年资助(万)
1	人卵泡闭锁调控机制的研究	陈士岭 (刘以训)	1997.5~1999.5	1.0
2	泛素系统降解蛋白质在小鼠精子细胞变态过程中的作用	王永潮 (陈大元)	1997.9~1999.8	2.0
3	人细胞滋养层中金属蛋白酶表达调节机制的研究	朴允尚	1997.5~1999.4	2.0
4	应用反义技术研究细胞因子对胚胎植入的作用机制	曹灵芳 (段恩奎)	1997.5~1999.4	2.0

### 98 年新申请课题:

1	异种哺乳动物间核移植的研究	李劲松 (陈大元)	1998.5~2000.4	2.0
2	RU486 用于紧急避孕对子宫内膜整合素及 MUC-1 蛋白表达的影响	郑淑蓉 (王妮)	1998.1~1999.12	2.0
3	着床过程 CAM 的动态研究	李 宾 (王妮)	1998.4~1999.4	2.0
4	RU486 对早孕恒河猴胎盘形成的影响	王训立 (刘以训)	1998.6~1999.12	1.0
5	纤蛋白溶酶原激活因子和抑制因子对精子功能的影响	邹如金 (刘以训)	1998.1~2000.2	2.0
6	小鼠附植启动过程中雌激素与表皮生长因子(EGF)的作用及相互关系	赵兴绪 (段恩奎)	1998 ~2000	2.0
7	肾素血管紧张素系统对卵母细胞成熟的调控研究	夏国良 (于红)	1998.4~2000.4	2.0
8	大鼠卵巢颗粒细胞凋亡机制的分子生物学研究--颗粒细胞凋亡与线粒体机能、bcl-2、EGF 及其受体基因表达的相互关系	于晓光 (祝诚)	1998.8~2000.8	2.0
9	受精机理及生殖工程研究	陈大元	1998.4~1999.4	4.3
10	精卵发生和黄体萎缩的基因调控	刘以训	1998.4~1999.4	4.3
11	动情期及早期妊娠金属蛋白酶在大鼠卵巢、子宫中的表达	祝 诚	1998.4~1999.4	4.2
12	滋养层组织金属蛋白酶的表达调控	朴允尚	1998.4~1999.4	4.2
13	细胞因子和整合素对植入的调节机理	段恩奎	1998.4~1999.4	4.0
14	细胞因子对胚胎着床的作用机理及其应用	于妮	1998.4~1999.4	3.5
15	子宫内膜血管易变性调节机制的探讨	于红	1998.4~1999.4	3.5

## 受精 (PART II)

1. Sun Qing-Yuan, Liu Hui and Chen Da-Yuan (1997) Calcium-independent, egg age-dependent parthenogenic activation of mouse eggs by staurosporine. *Journal of Reproduction and Development* 43: 189-197.-----77
2. Sun Qing-Yuan, Wang Wei-Hua, Hosoe Misa, Taniguchi Toshiaki, Chen Da-Yuan and Shioya Yasuo (1977) Activation of protein kinase C induces cortical granule exocytosis in a  $Ca^{2+}$ -independent manner, but not the resumption of cell cycle in porcine eggs. *Development, Growth & Differentiation* 39: 523-529.-----86
3. Sun Qing-Yuan, Gao Shao-Rong and Chen Da-Yuan (1997) The mechanism of sperm head transformation during mouse oocyte fertilization in vitro. *动物学报* 43: 382-389.-----93
4. 刘灵, 孙青原, 段崇文, 刘辉, 宋祥芬, 钱菊汾, 陈大元 (1997) 小鼠球形精子细胞带下受精研究. *中国科学 (C 辑)* 27: 145-150.-----102
5. Liu Ling, Sun Qing-Yuan, Duan Chong-Wen, Liu Hui, Song Xiang-Fen, Qian Ju-Fen and Chen Da-Yuan (1997) Subzonal fertilization of mouse round spermatid. *Science in China (Series C)* 40: 152-158.
6. 刘灵, 孙青原, 段崇文, 刘辉, 宋祥芬, 钱菊汾, 陈大元 (1997) 小鼠初级精母细胞带下受精的研究. *科学通报* 13: 1432-1435.-----115
7. Gao Shao-Rong, Sun Qing-Yuan, Huang Zhen-Yong and Chen Da-Yuan (1997) Effects of protein synthesis on the maturation of mouse oocytes in vitro. *Developmental & Reproductive Biology* 6: 1-6.-----119
8. 李明文, 刘辉, 孙青原, 焦日, 庄大中, 宋祥芬, 孙青原, 陈大元 (1997) 小鼠卵母细胞成熟和受精过程中  $Ca^{2+}$  分布变化的研究. *动物学报* 43: 80-84.-----125
9. 李明文, 焦日, 胡国俊, 刘辉, 庄大中, 陈大元 (1997) 钙通道阻断剂对豚鼠精子顶体反应的影响. *中国兽医学报* 17: 230-233.-----132
10. 温占强, 郑光美, 宋祥芬, 孙青原, 陈大元 (1997) 黄腹角雉精子超显微结构的研究. *动物学报* 43: 127-132.-----136
11. 段崇文, 胡国俊, 陈大元 (1997) 小鼠精子附睾成熟过程中  $Ca^{2+}$  及  $Ca^{2+}$ -ATPase 的研究. *电子显微学报* 16: 25-30.-----143
12. 段崇文, 胡国俊, 陈大元 (1997) 小鼠精子获能及顶体反应过程中  $Ca^{2+}$  及  $Ca^{2+}$ -ATPase 的研究. *电子显微学报* 16: 87-92.-----149
13. Chen Da-Yuan, Song Xiang-Fen, Duan Chong-Wen, Li Ming-Wen, Sun Qing-Yuan, Liu Hui, Zhang An-Ju, Ye Zhi-Yong, Li Shao-Chang, Yu Jian-Qiu, Zhang Fu-Xiang, Feng Wen-He, Zhong Shun-Long, He Guang-Xi, Song Yun-Fang and Fei-Li-Song (1997) Applying bases for "double control" artificial breeding of giant panda in captivity. *Chinese Science Bulletin* 42: 775-779.-----155
14. Sun Qing-Yuan, Lurisa Ayala, Rubinstein Sara and Breitbart Haim (1998) Protein kinase inhibitors induce the interphase transition by inactivating mitogen-activated protein kinase in mouse eggs. *Zygote* 6: 277-284.-----160

14. 高绍荣, 胡国俊, 段崇文, 刘辉, 韩之明, 宋祥芬, 陈大元 (1998) 抗精子单克隆抗体6B10的免疫定位及其相应抗原分析. 中国科学 (C 辑) 28: 548-553.-----168
15. 李劲松, 刘辉, 王敏康, 廉莉, 李厚达, 陈大元 (1998) 电刺激诱导兔卵孤雌活化的研究. 上海实验动物科学 18: 133-136.-----174

### 着床与胎盘 (PART III)

1. Li Rong-Hao and Zhuang Lin-Zhi (1997) The effects of growth factors on human normal placental cytotrophoblast cell proliferation. Human Reproduction 12: 830-834.-----177
2. 刘以训, 胡召元, 邹如今, Ockleford CD (1997) 人和恒河猴胎盘基分泌 tPA 和 PAI-1 部位的鉴定. 科学通报 42: 1324-1326.-----182
3. 曹宇静, 曾国庆 (1997) 层粘连蛋白及其肽段对小鼠胚泡粘附和扩展的作用. 动物学报 43: 85-89.-----185
4. 曹咏清, 陈幼珍, 张富春 (1997) 免胚泡肽合成片段及其与着床有关的生理功能. 生理学报 49: 562-568.-----191
5. 陈忠科, 焦丽红, 阎建设, 王红 (1997) 妊娠早期胎盘绒毛中肾素活性及其分泌的研究. 生理学报 49: 463-466.-----198
6. 张春雨, 曹宇静, 段恩奎, 曾国庆 (1997) 细胞表面半乳糖基转移酶介导外胎盘锥扩展和次滋养层巨细胞迁移的证据. 生殖医学杂志 6: 78-83.-----202
- Zhang Chun-Yu, Cao Yu-Jing, Duan En-Kui and Zeng Guo-Qing (1997) The role of cell surface  $\beta$ 1,4-galactosyltransferase during ectoplacental cone outgrowth on laminin. Journal of Reproductive Medicine 5: 98-104.
7. 曹宇静, 蒋广泰, 曾国庆 (1997) 蛋白酶抑制剂对小鼠胚泡着床的作用. 动物学研究 18: 93-98.-----215
8. 赵炳顺, 张沅, 邹继超, 张永莲 (1997) 羟泰米酚对着床前小鼠子宫雌激素受体基因表达的调节作用. 动物学报 43: 309-314.-----221
9. 朱艳, 陈贵安, 冯强, 武淑英, 刘以训 (1997) 米非司酮对早孕蜕膜中纤溶酶原激活及抑制因子及其尿激酶受体的分布与表达的影响. 生殖医学杂志 6: 147-151.-----227
10. 曾国庆, 蒋广泰 (1997) 层粘连蛋白活性肽段对小鼠胚泡着床的影响. 动物学报 43: 221-222.-----231
11. 朱艳, 陈贵安, 冯强, 武淑英, 刘以训 (1997) 纤溶酶原激活及抑制因子在人蜕膜腺体与间质细胞中的生成及调节. 生殖医学杂志 6: 99-103.-----233
12. 胡召元, 刘以训, Ockleford CD (1997) 纤溶酶原激活及抑制因子在胎膜组织中的免疫定位的研究. 生殖与避孕 17: 245-246.-----238
13. 黄威权, 张崇理, 逢新宇, 孙岚 (1997) 人胎盘绒毛 5-羟色胺受体的显微亚显微定位及定量研究. 科学通报 42: 233-239.-----241



- Huang Wei-Quan, Zhang Chong-Li, Di Xin-Yu and Sun Lan (1998) Microscopic and ultramicroscopic localizations and quantitative analysis of 5-HT receptors in human placentas. Chinese Science Bulletin 43: 804-809.
14. Feng J, Woessener Jr and Zhu C (1998) Matrilysin activity in the rat uterus during the oestrous cycle and implantation. Journal of Reproduction and Fertility 114: 347-350.-----254
15. Wang XQ, Zhu ZM, Fenderson BA, Zeng GQ, Cao YJ and Jiang GT (1998) Effects of monoclonal antibody directed to Le<sup>x</sup> on implantation in the mouse. Molecular Human Reproduction 4: 295-300.-----258
16. 张春雨, 段恩奎, 曹宇静, 曾国庆 (1998) 焦点粘着激酶在小鼠外胎盘锥体外扩展中的表达、分布和功能. 科学通报 43: 1527-1532.-----264
- Zhang Chun-Yu, Duan En-Kui, Cao Yu-Jing and Zeng Guo-Qing (1998) Expression, distribution and function of the focal adhesion kinase (pp125FAK) during murine ectoplacental cone outgrowth in vitro. Chinese Science Bulletin 43: 1473-1480.
17. 王晓琦, 刘煜, 段恩奎, 曾国庆 (1998) Le<sup>x</sup> 对小鼠胚泡和子宫上皮细胞分泌 MMPs 的影响. 科学通报 43: 1292-1295.-----278
- Wang Xiao-Qi, Liu Yu, Duan En-Kui and Zeng Guo-Qing (1998) Effects of blocking Le<sup>x</sup> Oligosaccharide on cell surface to MMPs secreted by blastocysts and epithelial cells in mouse in vitro. Chinese Science Bulletin 43: 1461-1465.
18. 王晓琦, 曹宇静, 曾国庆 (1998) 在共培养体系中单克隆抗体 AH<sub>6</sub> 对小鼠胚泡着床的影响. 动物学报 44: 443-449.-----287
19. 周祖平, 郑建建, 焦丽红, 张崇理 (1998) 脱氢表雄酮对人早期胎盘孕酮分泌的影响. 动物学报 44: 341-346.-----294
20. Li Bin, Zhou Chun-Xi, Wang Li-Ming, Wang Ni, Chen You-Zhen and Cao Yong-Qing (1998) Study on dynamic changes of ICAM-1 in mouse PBL and EC/DC at peri-implantation stage. Developmental and Reproductive Biology 7: 9-14.-----300
21. 陈浩宏, 顾芝萍, 游根娣, 庄临之 (1998) 人蜕膜细胞培养作为抗早孕药物筛选模型的研究. 上海铁道大学学报 19: 1-4.-----306
22. 陈毅军, 冯强, 刘以训 (1998) 组织型纤溶酶原激活及抑制因子在孕鼠子宫中的表达. 基础医学与临床 43: 123-126.-----310
23. Zhang Chun-Yu, Cao Yu-Jing and Zeng Guo-Qing (1997) Studies of murine ectoplacental cone cells interaction with laminin. Proceedings of Beijing Symposium on Fertility Regulation: Present and Future. 2-59-69. -----314
24. Zhang Chong-Li, Shen Wei-Bin, Huang Wei-Qin, Zhang Rong-Qing, Chen Li-Ren, Du Wei, Jiao Li-Hong and Shi Jia (1997) The presence and function of neurotransmitters in the human early placenta. Proceedings of Beijing Symposium on Fertility Regulation: Present and Future. 2-70-80.-----325
- \*25. Cao Yong-Qing, Chen You-Zhen and Zhang Fu-Chun (1996) The synthetic fragment of rabbit blastocyst peptide 2 and its physiological significance relevant to the mechanism of implantation. Journal of Reproductive Medicine 5: 105-110.-----344

- \*26. Cao Yong-Qing and Chen You-Zheng (1996) The effect of human recombinant interferon gamma (hrIFN- $\gamma$ ) on hCG secretion of trophoblast and proteinsynthesis of decidual tissue in vitro. *Reproduction & Contraception* 7: 73-80.-----350

## 其它内容 (PART IV)

1. Liu YX, Hu ZY, Liu K, Byrne S, Zou RJ, Ny T, d'Lacey C and Ockleford CD (1998) Localization and distribution of tissue type and urokinase type plasminogen activators and their inhibitors type 1 and type 2 in human and rhesus monkey fetal membranes. *Placenta* 19: 171-180.-----359
2. Mu Xiao-Min, Young Win-Jing, Liu Yi-Xun, Uemura Hiroji and Chang Chawshang (1998) Induction of an intronic enhancer of the human ciliary neurotrophic factor receptor (CNTFR $\alpha$ ) gene by the TR3 orphan receptor. *Endocrine* 9: 27-32.-----369
3. 穆小民, 刘以训, 张传祥 (1998) 孤儿受体 TR3 与人 CNTF 受体基因中顺式元件作用机制的研究. *中国生物化学与分子生物学报* 14: 485-492.---375
4. 金花淑, 黄威权, 张金山, 文永植, 张崇理 (1998) 大鼠颌下腺促性腺激素释放激素及其 mRNA 的免疫组织化学与原位杂交研究. *动物学报* 44: 186-189.-----382
5. 邹爱民, 张崇理, 魏兴德 (1998) 妊娠期母体血浆与胎盘组织中去甲肾上腺素含量的研究. *北京医学* 20: 117-118.-----386
6. 宋济范, 刘先菊, 王思泽, 王妮 (1998) 人输卵管中与植物凝集素 BS-1 结合糖蛋白初步鉴定. *中国计划生育学杂志* 7: 298-300.-----388
7. 邹爱民, 张崇理, 魏兴德 (1998) 妊娠期血浆与胎盘内 P 物质含量变化的研究. *中国厂矿医学* 2: 88-89.-----391

## (二) 综述及科普文章

1. 刘以训, 陈毅军, 冯强, 胡召元 (1997) 纤溶酶原激活及抑制因子在溶黄体过程中的作用. *科学通报* 42: 2026-2030.-----393
2. 张春雨, 段恩奎, 曾国庆, 刘以训 (1997) 细胞表面半乳糖基转移酶及其生物学功能. *生物化学与生物物理进展* 24: 518-564.-----398
3. 张春雨, 段恩奎, 曾国庆 (1997) 白血病抑制因子及其在胚胎发育和胚胎着床中的生理功能. *生理科学进展* 28: 240-242.-----402
4. 刘灵, 钱菊汾, 陈大元 (1997) 哺乳动物显微受精研究进展. *西北农业大学学报* 25: 89-94.-----405
5. 张春雨 (1997) 胚胎植入与肿瘤浸润转移的相似性. *生命科学* 9: 97-110.-----412

6. 朱艳, 陈贵安, 刘以训 (1997) 纤溶酶原激活、抑制系统及其在女性生殖中的作用. 生殖医学杂志 6: 186-189.-----416
7. 陈大元 (1997) 拯救大熊猫赢得主动, 保护大熊猫有了希望. 生命科学 9: 31-33.-----420
8. 陈大元 (1997) 显微受精与动物克隆的研究及进展.-----423
9. 庄临之 (1997) 迎接 21 世纪生命科学——计划生育的基础研究问题.-----427
10. 段恩奎 (1997) H-Y 抗原与动物性别控制技术. 《动物生殖调控》 P319-323.-----432
11. 穆小民, 刘以训 (1997) 核受体及其转录活化机制研究的新突破——辅活化子和辅阻遏子. 生物化学与生物物理进展 25: 222-226.-----437
12. 韩新兵, 曹咏清, 庄临之 (1998) 促性腺激素释放激素受体及其基因表达调控. 生理科学进展 29: 198-202.-----442
13. 陈大元 (1998) 显微授精技术与人类优生优育. 中国计划生育学杂志 8: 352-353.-----447
14. Chen Da-Yuan (1998) Clones in China. China Today, June 25-27.-----449
15. 刘冀珑 (1998) 钙生物学——一门方兴未艾的科学. 科学 8: 56-59.-----452
16. 刘冀珑 (1998) 有趣的钙生物学. 中国科技画报 5: 23-26.-----456

\*: 朱载入 95-96 论文集的论文

## Prolactin delays gonadotrophin-induced ovulation and down-regulates expression of plasminogen-activator system in ovary

Yi-Xun Liu<sup>1,3</sup>, Xiao-Rong Peng<sup>2</sup>, Yi-Jun Chen<sup>1</sup>, Whitney Carriço<sup>2</sup>, and Tor Ny<sup>3</sup>

<sup>1</sup>State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China and

<sup>2</sup>Department of Medical Biochemistry and Biophysics, Umeå University, S-901 87 Umeå, Sweden

<sup>3</sup>To whom correspondence should be addressed

This study was conducted to determine whether prolactin (PRL) suppresses gonadotrophin-induced ovulation and disturbs the co-ordinated gene expression of tissue type plasminogen activator (tPA) and plasminogen activator inhibitor type-1 (PAI-1) in rat ovary. Immature female rats were injected with 10 IU pregnant mare's serum gonadotrophin to stimulate follicle growth, and 48 h received different doses of prolactin followed by 7 IU human chorionic gonadotrophin (HCG). The oviducts were examined for the presence of ova, and the amounts of tPA and PAI-1 mRNA present in the ovary were measured at various times after the hormone treatment. PRL had no significant effect on ovarian weight but caused a dose-dependent decrease in ovulation number. In the control animals receiving HCG alone,  $13.3 \pm 1.3$  (mean  $\pm$  SEM) ova/oviduct were found; while in animals receiving HCG plus 50, 100 or 200  $\mu$ g PRL, the ovulation number was dose-dependently suppressed by 53.6, 66.9 and 76% respectively at 18 h after treatment. PRL suppression of HCG-induced ovulation was time-dependent. By 24 h after treatment, the number of ova in the oviducts in HCG- and HCG plus PRL-treated groups was not significantly different. PRL also suppressed HCG-induced tPA gene expression in a dose- and time-dependent manner. At all time points examined, tPA mRNA content of whole ovaries and granulosa cells (GC) in PRL-treated groups was lower than in the HCG-treated controls. The activities of PAI-1 in ovarian extracellular fluid (OEF) and PAI-1 mRNA in the theca-interstitial cells (TI) in the PRL-treated groups were higher than in the HCG-treated controls. The highest stimulation by PRL of PAI-1 activity in OEF and of PAI-1 mRNA in TI was observed at 9 h and 6 h after HCG treatment respectively. The localization of tPA and PAI-1 antigens in the ovaries was consistent with changes in the mRNA and activity levels. These data suggest that PRL temporarily delays, but does not completely inhibit, HCG-induced ovulation, which may be caused by a suppression of PA-mediated proteolysis.

**Key words:** ovulation/plasminogen activator inhibitor type-1/prolactin/tissue type plasminogen activator

### Introduction

Hyperprolactinaemia, or elevation of prolactin (PRL) secretion during lactation, is associated with anovulation (McNeilly *et al.*, 1982). Clinical studies have indicated that PRL acts directly on developing human follicles to inhibit ovarian steroidogenesis, follicular maturation and ovulation (McNatty *et al.*, 1974; McNatty, 1979; Jacobs *et al.*, 1976; McNeill, 1987). Specific PRL receptors have been found in the ovary of several mammalian species (Saito and Saxena, 1975). PRL treatment *in vitro* has been shown to cause a decrease in ovarian aromatase activity (Tsai-Morris *et al.*, 1983; Hu and Liu, 1995). Using an *in-vitro* perfused rabbit ovarian system, PRL was found to inhibit human chorionic gonadotrophin (HCG)-induced ovulation (Hamada *et al.*, 1980), indicating that PRL acts directly on the ovary by interfering with the mechanisms causing rupture of the follicle. Results from many studies indicate that the plasminogen activator system is instrumental for the induction of ovulation: (1) there is a transient increase in tissue type plasminogen activator (tPA) activity in the rat ovary just prior to ovulation (Liu *et al.*, 1987); (2) plasminogen is present in follicular fluid, and plasmin has been shown to weaken the follicle wall *in vitro* (Beers, 1975); (3) intrabursal injection of protease inhibitor, and antibodies against tPA partially blocks gonadotrophin-induced ovulation in rats (Retch *et al.*, 1985; Tsafiri *et al.*, 1989; Guerre *et al.*, 1991); (4) not only PAs, but also the specific PA inhibitor PAI-1, are regulated during the periovulatory period (Liu, 1988; Liu *et al.*, 1991); (5) *in-situ* localization studies revealed that tPA and PAI-1 are expressed in a cell specific and time-co-ordinated fashion (Chun *et al.*, 1992; Peng *et al.*, 1993). These data suggest that proteolytic activity provided by tPA and modulated by PAI-1 may cause a directed proteolysis leading to rupture of the selected follicle during ovulation.

Using *in-vitro* perfused viable rabbit ovaries, it has been shown that PRL causes a decrease in the PA activity of mature follicles (Yoshimura *et al.*, 1990, 1992). The objective of this study was to examine the effect of PRL on ovulation, and the expression of tPA and PAI-1 in rat ovaries.

### Materials and methods

#### Materials

McCoy's 5a medium (modified, without serum), penicillin-streptomycin solution, L-glutamine and fetal calf serum were purchased from Gibco (Paisley, UK); pregnant mare's serum gonadotrophin (PMSG), was obtained from Sigma Chemical Company (St Louis, MO, USA); acrylamide N,N'-methylene-bis-acrylamide, sodium docteryl sulphate tetramethylene diamine (TEMED), ammonium persulphate, C-mix-

are brilliant blue were from Bio-Rad Laboratories (Richmond, CA, USA). Nylon filters were obtained from Amersham (UK); urokinase type plasminogen activator was obtained from Serono S.A. (Aubonne, Switzerland); plasminogen was obtained from BioPuri (Rimel, Sweden); human fibrinogen was purchased from KabiVitrum (Stockholm, Sweden) and further purified by ethanol precipitation as described by Blomback and Blomback (1956). T7 RNA polymerase was obtained from Promega (Madison, WI, USA) and [ $\gamma$ - $^{32}$ P] UTP was from Amersham (UK). Restriction enzymes were purchased from Boehringer Mannheim (Bromma, Sweden) and collagenase was obtained from Worthington Biochemical Corporation (New Jersey, USA). Ovine PRL (NIDDK-PRL-15, 31 IU/mg) was obtained from National Institute of Diabetes, Digestive and Kidney Diseases; Human chorionic gonadotropin (HCG CR-127, 14 900 IU/mg) was obtained from National Institute of Child Health and Human Development (NICHD, USA).

#### Animal treatment

Immature female rats of the Sprague-Dawley strain (21–22 days old) were obtained from Alab Laboratorietjänst AB (Stockholm, Sweden) or Institute of Zoology, Chinese Academy of Sciences (Beijing, China), and were fed with chow and water *ad libitum*. A 14:10 h light-dark cycle was maintained with the light cycle initiating at 0600 h. The animals were injected s.c. with a superovulating dose (10 IU) of pregnant mare's serum gonadotropin (PMSG) to stimulate follicle growth. Forty-eight hours later, 7 IU HCG was given to induce ovulation. In the indicated groups 50–200  $\mu$ g PRL in 100  $\mu$ l of saline was also given 15 min before HCG injection. The animals were killed at 6, 9, 12, 16, 18 and 24 h after injection of HCG or HCG plus various doses of PRL. The oviducts were examined for the presence of ova. The ovaries were isolated, and the ovarian extracellular fluid (OEF), granulosa cells and theca-interstitial tissue were prepared for tPA, PAI-1 mRNA and activity measurement (Liu *et al.*, 1991).

#### Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE)

PA and PAI-1 activities in the samples were fractionated by SDS-PAGE according to Laemmli (1970). Before electrophoresis, the samples were adjusted to 2.5% SDS (for PA-activity assay) and 2.5% SDS and 12.5 mM DTT (for PAI-1 activity assay). Electrophoresis was performed at 50 V until the dye front reached the bottom of the gel (~16 h). After electrophoresis, gels were incubated 2×45 min in 2.5% (v/v) Triton X-100 to remove SDS in the gel, rinsed with distilled water and applied onto the surface of a fibrin-agar indicator gel.

#### Fibrin overlay and reverse fibrin autography assay

The fibrin-agar indicator gel was prepared as previously described (Granelli-Piperno *et al.*, 1978). The fibrin-agar gel contained 50  $\mu$ g/ml plasminogen, 2.4 mg/ml fibrinogen, and 0.5 U/ml thrombin for generation of fibrin as the substrate for plasmin. After electrophoresis, the gel was laid onto the fibrin-agar indicator gel and incubated at 37°C in a humid chamber until the lysis zones became visible, indicating the presence of PAs. For detection of the PAI-1 activity, the samples were analysed by a reverse fibrin autograph (Erickson *et al.*, 1984). In addition to plasminogen, fibrinogen and thrombin, RFA indicator gel contained 0.01 U/ml urokinase to allow autolysis. The development of an opaque, lysin-resistant zone indicated the presence of PAI-1 activity.

#### Synthesis of RNA and DNA probes

A rat PAI-1 cDNA was cloned into the pBluescript vector (Zehnbach and Geethner, 1988) and a 400 bp of EcoRI fragment from the rat tPA cDNA clone lambda 15 (Niy *et al.*, 1988) was subcloned into

pGEM-1 vector. Both vectors were linearized by Hind III and used as templates for probe synthesis using an *in-vitro* transcription system (Promega, Madison, WI, USA). A 400 bp tPA probe complementary to the 5' region of rat tPA mRNA, and a 576 bp PAI-1 probe complementary to 3'-untranslated region of PAI-1 mRNA were obtained using T7 and T3 promoters, respectively. The  $^{32}$ P-labelled antisense RNA probes were tested for specificity by hybridization to ovarian total RNA fractionated by formaldehyde agarose gel electrophoresis and blotted to nylon filters (Thomas, 1980; Manattis *et al.*, 1982). The probes were found to be specific for tPA and PAI-1 mRNA, since they hybridized only to specific mRNA species with the size corresponding to PA (Niy *et al.*, 1988), and PAI-1 mRNA (Zehnbach and Geethner, 1988). A 250 nucleotide long single stranded  $\beta$ -actin DNA probe was prepared by primer extension as described (Ohlsson *et al.*, 1988).

#### Preparation of total RNA

Cytoplasmic RNA from granulosa cells was prepared using the NP-40 method (Manattis *et al.*, 1982). Total RNA was prepared from whole ovaries and from theca-interstitial tissue using CsCl gradient density separation method (Peng *et al.*, 1993). For hybridization analysis, total RNA was fractionated by agarose gel electrophoresis in the presence of formaldehyde (Holmes *et al.*, 1986) and transferred to nylon filters (Thomas, 1980) or immobilized directly by using a slot blot filtration apparatus (Schlesinger & Schull, Dassau, Germany). Serial dilution of RNA from each sample was applied to nylon filter for hybridization with PAI-1, tPA and  $\beta$ -actin probes. Nylon filters were cross-linked using a Stratilinker (Stratagene, Sweden), and prehybridized in 50% formamide, 5× saline sodium citrate (SSC), 8× Denhardt's solution (1.6 mg/ml Ficoll, 1.6 mg/ml polyvinylpyrrolidone, 1.6 mg/ml BSA), 0.1% SDS, 10 mM EDTA, 25 mM Tris-HCl pH 7.0, 250  $\mu$ g/ml heat denatured herring sperm DNA, 250  $\mu$ g/ml yeast tRNA at 62°C for 2 h. The hybridization was carried out in the same solution containing 2.5×10<sup>6</sup> c.p.m./ml of each probe for 16 h at 64°C. The filters were washed in 2×SSC, 0.1% SDS twice for 15 min at room temperature and followed by two washes in 0.1×SSC, 0.1% SDS for 40 min at 66°C. Hybridization using the  $\beta$ -actin probe was performed at 42°C as described (Ohlsson *et al.*, 1988). After hybridization, the filters were analysed by autoradiography (Molecular Dynamics, Sweden), or were exposed to phosphorographic films.

#### In-situ hybridization

The ovaries left for *in-situ* hybridization were fixed in Bouin's fluid and embedded in paraffin prior to sectioning (4  $\mu$ m), according to standard procedures. The deparaffinized sections were treated with 8 mg/ml proteinase K (E. Merck, Darmstadt, Germany) for 10 min and washed in PBS for 5 min. Sections were then fixed in 4% paraformaldehyde in PBS for 5 min and washed in PBS for 10 min. Before hybridization, the sections were dehydrated through a graded ethanol series and allowed to air-dry. The sections were prehybridized with 50% formamide and 2×SSC for 2 h at room temperature, then hybridized overnight with digoxigenin (DIG)-labelled tPA RNA probe in hybridization buffer (10 mM Tris-HCl, pH 7.5, 2×SSC, 50% deionized formamide, 1×Denhardt's, 2.5 mM DTT, 5% dextran sulphate, 250  $\mu$ g/ml yeast tRNA, and 0.5% SDS) at 48°C. After hybridization, the sections were thoroughly washed in 2×, 1×, and 0.1×SSC, each for 2×15 min at 40°C. The sections were then rinsed in DIG buffer 1 (0.1 M maleic acid, 150 mM NaCl, pH 7.5) for 5 min, and blocked with 1% blocking reagent in buffer 1. The sections were incubated with alkaline phosphatase conjugated anti-DIG IgG diluted 1:200 in buffer 1 containing 1% blocking reagent for 2 h washed in PBS for 3×5 min. The bound antibody was detected by a standard immuno-alkaline phosphatase reaction using 5-bromo-4-

Table I. Dose-dependent inhibition of HCG-induced ovulation by prolactin in PMSG-primed immature rats

$\mu\text{g PRL}$	No. animals	Ovarian wt	No. ovulating rats	No. ova/ovary	% inhibition
0	13	39.2 $\pm$ 1.7	13	13 $\pm$ 1.3	0
50	8	39.2 $\pm$ 2.6	6	6.3 $\pm$ 1.0*	47.4
100	10	40.6 $\pm$ 2.9	7	4.4 $\pm$ 0.8*	66.9
200	17	34.5 $\pm$ 2.3	8	3.2 $\pm$ 0.8*	76.0

\* $P < 0.01$  compared with control (0 PRL). Results are expressed as mean  $\pm$  SEM.

HCG = human chorionic gonadotrophin; PMSG = pregnant mare's serum gonadotrophin.

Table II. Time-dependent inhibition of HCG-induced ovulation by prolactin in PMSG-primed immature rats

Hours after HCG treatment		No. rats	No. ovulating rats	No. ova/ovary	% inhibition
injection					
12	HCG	10	2	2.0 $\pm$ 1.2	80
	HCG + PRL	10	1	0.4 $\pm$ 1.3*	
16	HCG	6	4	9.3 $\pm$ 3.1	100
	HCG + PRL	6	0	0**	
18	HCG	9	8	11.0 $\pm$ 2.7	90
	HCG + PRL	9	2	1.1 $\pm$ 0.9**	
20	HCG	7	7	12.6 $\pm$ 2.1	79
	HCG + PRL	7	3	2.7 $\pm$ 1.3**	
24	HCG	14	13	13.9 $\pm$ 1.3	7.1
	HCG + PRL	14	12	12.9 $\pm$ 1.8	

\* $P < 0.05$ , \*\* $P < 0.01$  compared with control group (HCG alone).

HCG = human chorionic gonadotrophin; PMSG = pregnant mare's serum gonadotrophin.

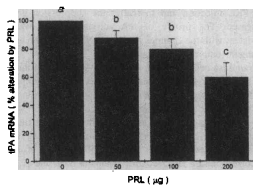


Figure 1. Dose-dependent effect of prolactin on ovarian tissue plasminogen activator (tPA) mRNA levels. Ovarian samples were obtained following treatment with human chorionic gonadotrophin (HCG) as described in Materials and methods. The total RNA was analysed for tPA and  $\beta$ -actin mRNA. The relative amount of tPA mRNA was estimated by phosphorimager and normalized against the corresponding relative amounts of  $\beta$ -actin mRNA. Results are expressed as mean  $\pm$  SEM. Different letters indicate significant difference between doses ( $P < 0.05$ ).

chloro-3-indolyl phosphate (BCIP) and nitro-blue tetrazolium (NBT) as substrate. The DIG RNA labelling kit and all the reagents used for DIG detection were from Boehringer Mannheim GmbH Biochemica (Mannheim, Germany).

#### Data analysis

All experiments measuring tPA and PAI-1 activity were repeated at least three times. A photographic record using dark-field illumination of one representative experiment is shown.

The relative amount of specific mRNA was determined by quantita-

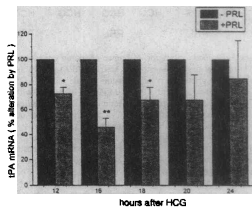


Figure 2. Time-dependent effect of prolactin (PRL) on ovarian tissue plasminogen activator (tPA) mRNA content. Ovarian samples were obtained following treatment with human chorionic gonadotrophin (HCG) and PRL for different time periods as described in Materials and methods. The total RNA was analysed for tPA and  $\beta$ -actin mRNA. The relative amount of tPA mRNA was estimated by phosphorimager and normalized against the corresponding relative amount of  $\beta$ -actin mRNA. Results are expressed as mean  $\pm$  SEM. \* $P < 0.05$ , \*\* $P < 0.01$  compared with corresponding control groups (Student's *t*-test).

tion by densitometric scanning of autoradiographic films, and was normalized against the corresponding relative amount of  $\beta$ -actin in the samples. The data are expressed as fold increase relative to the control group or as mean  $\pm$  SEM of at least three separate experiments. Data were analysed by one-way analysis of variance. Differences among groups were detected by Tukey's Multiple Comparison test (Sokal and Rohlf, 1981). Paired comparisons, when required, were

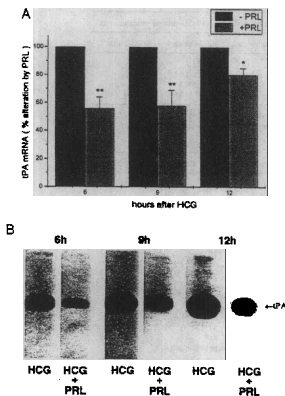


Figure 3. Prolactin inhibition of human chorionic gonadotropin (HCG)-induced tissue plasminogen activator (tPA) mRNA (A) and activity (B) in granulosa cells. Immature female rats (21–22 days old) were injected s.c. with 10 IU PMSG and 48 h later with 7 IU HCG, or HCG plus 200 µg of prolactin (PRL). At the indicated times the animals were killed. (A) Cytoplasmic RNA was obtained from the granulosa cells, was fractionated by agarose gel electrophoresis and hybridized with tPA and  $\beta$ -actin probes. The relative amount of specific mRNA was determined by densitometric scanning of autoradiographic films and normalized against corresponding relative amount of  $\beta$ -actin in the samples. The data are expressed as % of inhibition to the control (100%). \* $P < 0.05$ , \*\* $P < 0.01$  compared with corresponding control groups (-PRL) (Student's *t*-test). (B) tPA was extracted from  $0.5 \times 10^6$  granulosa cells, separated by SDS-PAGE, and analysed by fibrin overlay technique.

made by using Student's *t*-test. Differences were considered significant when  $P < 0.05$ .

## Results

### Dose- and time-dependent inhibition of HCG-induced ovulation by prolactin in PMSG-primed immature rats

To study the effect of PRL on ovulation, immature rats were injected s.c. with 10 IU PMSG in saline, and subsequently with 7.0 IU HCG alone or HCG plus the indicated doses of PRL. Eighteen hours after the hormone treatment the ovaries were removed and weighed and the oviducts were examined for presence of ova. As shown in Table I, PRL at all doses used had no significant effect on ovarian weight, but dose-

dependently decreased the number of ova present in the oviducts, by 47.4, 66.9 and 76%, at doses of 50, 100 and 200 µg PRL respectively.

To study whether the PRL inhibition of ovulation was time-dependent, ovulation was induced with 10 IU PMSG, followed by HCG in the presence and absence of PRL (200 µg). At the indicated times, the animals were killed, and the oviducts were examined for the presence of ova. As shown in Table II, PRL inhibition of ovulation was time-dependent. The average number of ova in the PRL-treated group was significantly decreased at 12, 16, 18 and 20 h after hormone treatment, but by 24 h the difference was not significant. This indicates that PRL did not completely inhibit, but temporarily delayed, HCG-induced ovulation.

### Prolactin inhibition of HCG-induced tPA mRNA content and activity in the ovary

To examine whether PRL inhibited the synthesis of tPA in the ovary, ovarian samples were analysed for tPA mRNA and activity. As shown in Figure 1, the ovarian tPA mRNA content induced by injection of HCG at 18 h was significantly decreased in a dose-dependent manner by treatment with PRL. At all time points during ovulation, the tPA mRNA levels were lower in the groups treated with PRL (200 µg) as shown in Figure 2. The PRL inhibition of HCG-induced tPA mRNA production in the ovary was most pronounced at 16 h after HCG and PRL injection and thereafter the suppression gradually decreased. The profile of tPA activity in the ovary was consistent with the changes in tPA mRNA during the hormone treatment (data not shown).

Granulosa cells produce the most tPA activity in the ovary, the tPA being secreted into the extracellular compartment (OEF). We therefore examined the changes in both tPA mRNA and tPA activity levels in granulosa cells and OEF at times preceding ovulation, 6, 9, and 12 h after hormone treatment. As shown in Figure 3A, the tPA mRNA content of granulosa cells in PRL-treated groups were 56, 58 and 80% of those of the corresponding HCG control groups at 6, 9, and 12 h. The PRL-induced decrease in tPA mRNA in granulosa cells was well correlated with the decrease in the cellular content of tPA activity (Figure 3B), and the tPA activity in OEF (data not shown).

### In-situ localization of HCG-induced tPA mRNA in the ovary

To study the effect of PRL on HCG-induced tPA mRNA localization in the ovary, non-radioactive in-situ hybridization was performed. As shown in Figure 4, tPA mRNA levels in granulosa cells induced by injection of HCG after 6 (A), 9 (C), and 12 h (E) were considerably inhibited by the co-injection of PRL after 6 (B), 9 (D), and 12 h (F).

### Effect of prolactin on PAI-1 expression in the ovary

PAI-1 is a rapidly secreted protein, barely detectable in the cell lysates. To estimate the PAI-1 activity in the extracellular environment, OEF was prepared from ovaries at 6, 9, and 12 h after HCG treatment, and the samples were analysed for PAI-1 activity by SDS-PAGE. As shown in Figure 5, at 6, 9, and 12 h after the hormone injection, PAI-1 activity in the

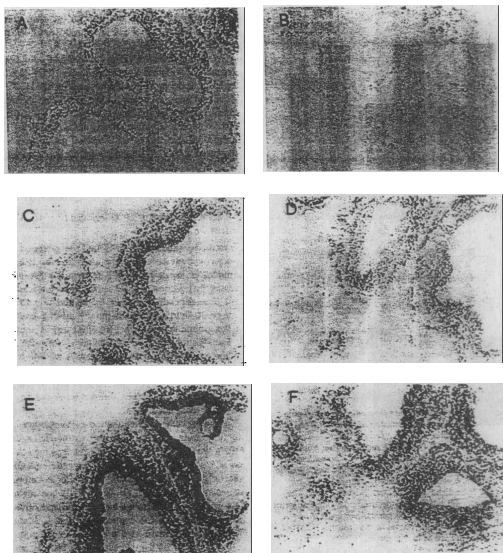


Figure 4. In-situ localization of tissue plasminogen activator (tPA) mRNA in ovarian cells. Immature female rats (21–22 days old) were injected s.c. with 10 IU pregnant mare's serum gonadotrophin (PMSG) and 48 h later with 7 IU human chorionic gonadotrophin (HCG), or HCG plus 200 µg of prolactin (PRL). After 6, 9 or 12 h, the animals were killed, the ovaries were removed and fixed in Bouin's fluid. Non-radioactive in-situ hybridization was performed as described in materials and methods. (A), (C) and (E): Localization of tPA mRNA in the ovaries 6, 9, and 12 h after HCG treatment respectively. (B), (D) and (F): Localization of tPA mRNA 6, 9 and 12 h respectively after treatment with HCG plus PRL.

OEF collected from the PRL-treated group was considerably higher than that in the HCG-treated group. The absolute PAI-1 activity in both groups was remarkably decreased by 12 h after the hormone treatment, perhaps due to a pre-ovulatory burst of tPA activity at this time point. The presence of multiple molecular weight forms is analogous to that found for PAI-1 secreted by rat HTC hepatoma cells (Zeheb *et al.*, 1987), and is mainly due to differences in glycosylation (Zeheb *et al.*, 1987; Liu *et al.*, 1991).

Theca-interstitial cells have been shown to secrete the most

PAI-1 activity in the ovary. To estimate the effect of PRL on PAI-1 gene expression in theca tissue, ovaries were punctured, granulosa cells were removed, and the residue tissue was examined for PAI-1 mRNA levels. As shown in Figure 6, the levels of PAI-1 mRNA were significantly higher in the groups receiving treatment with both HCG and PRL than in HCG-treated groups. The PAI-1 mRNA contents of the tissues of PRL-treated groups were 154, 120, and 135% of that in the corresponding HCG groups at 6, 9, and 12 h respectively after the hormone treatment.



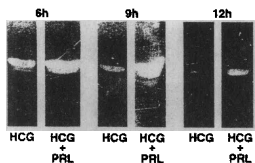


Figure 5. Prolactin stimulation of PAI-1 activity in ovarian extracellular fluid. Extracellular fluid (OEF) was obtained from the ovaries and fractionated by SDS-PAGE. PAI-1 activity in the gel was analysed by reverse fibrin autography as described in Materials and methods.

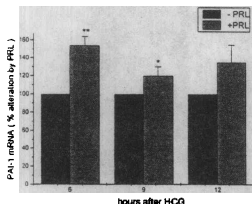


Figure 6. Prolactin stimulation of PAI-1 mRNA in theca-interstitial tissue. Theca-interstitial tissue was obtained from ovaries, following treatment with human chorionic gonadotropin (HCG) and prolactin (PRL) for various time periods, and after removal of granulosa cells, the tissues were analysed for PAI-1 mRNA. Results are expressed as mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$  compared with corresponding control group (-PRL) (Student's  $t$ -test).

## Discussion

We have demonstrated that treatment of PMSG-primed immature rats with PRL significantly inhibits and delays HCG-induced ovulation in a dose- and time-dependent manner. Further studies have suggested that PRL inhibition and delay of ovulation may be due to temporal suppression of gonadotropin-induced PA gene expression in granulosa cells on one hand, and stimulation of PAI-1 production on the other. These studies provide further evidence to show that PRL acts on the ovarian PA-PAI-1 system to interfere with mechanisms leading to ovulation. These findings are consistent with previous reports in mouse (Hu and Liu, 1993, 1995) and rabbit (Hamada *et al.*, 1980). Using a well designed in-vitro perfused rabbit ovary preparation, Hamada *et al.* (1980) demonstrated that addition of PRL to the perfusate significantly decreased HCG-induced ovulation. Yoshimura *et al.* (1992) further demonstrated that PA activity induced by HCG in the perfused rabbit ovary was significantly inhibited by the high doses of PRL in

the perfusate. That PRL, a compound that is known to inhibit ovulation, decreases both mRNA and activity levels of tPA activity in the ovary, further highlights the importance of the PA system in the ovary. These results strongly support the hypothesis that the PA system is the primary proteolytic enzyme responsible for follicle rupture (Beers, 1975; Strickland and Beers, 1976; Canipari and Strickland, 1985; Reich *et al.*, 1985; Liu *et al.*, 1987; Hsueh *et al.*, 1988). However, the precise mechanism by which PRL affects tPA mRNA and tPA activity is unknown.

Differences between the mouse and rat have been demonstrated. Mouse ovary mainly expresses uPA (Canipari *et al.*, 1985; Liu *et al.*, 1992; Leonardsson *et al.*, 1995), a lesser amount of tPA (Liu *et al.*, 1992; Leonardsson *et al.*, 1995) and various types of matrix metalloproteinases (K. Liu *et al.*, unpublished data). No PAI-1, but  $\alpha_2$ -antiplasmin was detected in mouse granulosa cells (Liu *et al.*, 1992). Both uPA and tPA in the mouse ovary were stimulated by the injection of PMSG/HCG and reached maximum levels just prior to ovulation (Liu *et al.*, 1992), implying that both tPA and uPA may play a role in the processes of follicular rupture in the mouse. Recent studies on autologous plasminogen activator gene replacement in mice have shown that in mice with a deficiency of tPA, uPA or PAI-1, the litter sizes and the life-span were normal (Carmeliet *et al.*, 1994). However, in mice with combined deficiencies of tPA and uPA, fertilization was significantly reduced (Carmeliet *et al.*, 1994; Leonardsson *et al.*, 1995). In-vivo and in-vitro studies also showed that in gonadotropin-treated mice, PRL inhibited both tPA and uPA, and also ovulation (Hu *et al.*, 1993, 1995). These data suggest that other proteases may provide proteolytic activity sufficient for follicular rupture in the absence of PA in the mouse. A functionally redundant mechanism for plasmin formation may operate in mouse ovary during gonadotropin-induced ovulation. The PA, together with other proteases, generate the proteolytic activity required for follicular wall degradation.

PRL is the primary pituitary hormone known to be involved in stimulating milk production. Marshall *et al.* (1986) observed high levels of tPA activity present in the colostrum and transitional milk, which was inversely related to the duration of lactation. However, it is most likely that the tPA activity found in the milk is synthesized in the breast tissue and is not derived from other sources. Both normal and malignant breast tissue have been shown to carry various steroid receptors (Isotalo *et al.*, 1983; Reiner *et al.*, 1984), and the increased cortisol, oestrogen and progesterone secretion during early lactation may be responsible for the increase in tPA production (Isotalo *et al.*, 1983; Bulter *et al.*, 1983; Huft and Lippman, 1984; Reiner *et al.*, 1994). No evidence has been found that prolactin is responsible for the increase in tPA activity in the breast tissue.

There is abundant evidence to show that the ovary is also one of the primary target tissue for PRL. Specific receptors for PRL have been found in granulosa cells and other compartments of the ovary (Saito *et al.*, 1975). There have been several reports that increased levels of PRL in plasma are associated with reduced follicular maturation and steroid production (Jacobs *et al.*, 1976; McNeilly *et al.*, 1982). However,