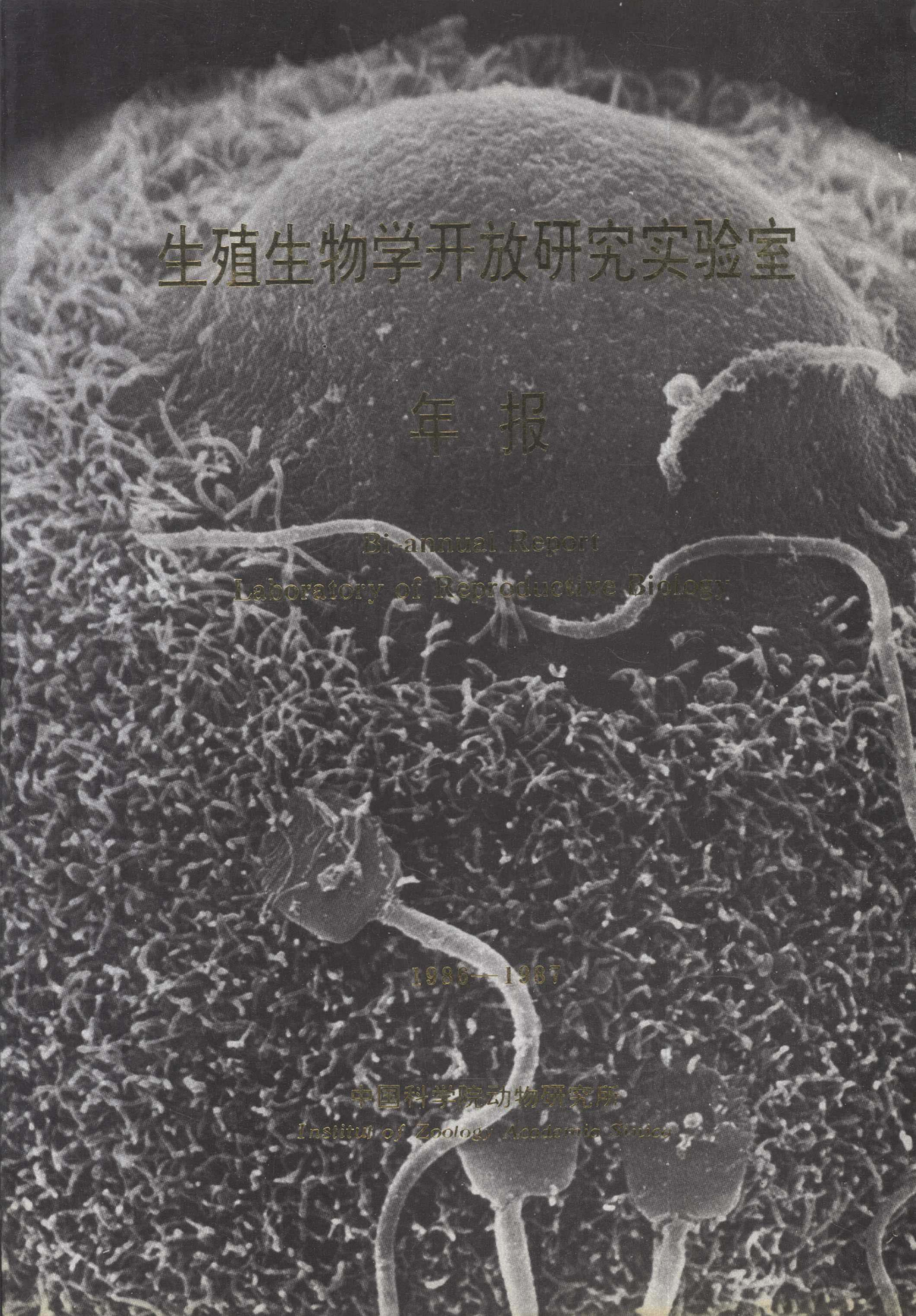

生殖生物学开放研究实验室

年报

Bi-annual Report

Laboratory of Reproductive Biology

1986—1987

中国科学院动物研究所

Institute of Zoology, Academia Sinica

目　　录

封面：大熊猫体外获能精子穿卵(仓鼠卵)的扫描电镜照片。

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The front cover: Egg penetration (in hamster egg) of *in vitro* capacitated Giant Panda sperm.

生殖生物学研究的简要回顾

生殖是生命之本，没有生殖过程，生物有机体就不能繁衍，系统进化就不可能出现。从社会和经济的观点来看，生殖研究的重大意义在于它不仅关系到人口的控制，而且也关系到改善动物蛋白产物的数量和质量。

生殖生物学是综合研究生殖的科学。作为生命科学中一门独立的学科，它首先出现在20世纪中叶。生殖生物学发展非常迅速，它与自然科学其他分支学科的发展一样，都是首先从描述性研究开始，然后是实验性探讨，最终形成一门综合性的生命科学，着重在分子水平上研究生殖的基本规律。

从历史上看，生殖生理学可以说是自然科学中第一个探讨生殖现象的学科。本世纪初，*Starling* 将激素的概念引入生理学后，生殖生理学的研究取得了很快的发展。到30年代，很多激素的提取、纯化和特性分析取得了很大进展，与此同时，人们也成功地进行了性类固醇的化学合成。这些进展使生殖生理学家们认识到：激素在生殖活动的调节中起着极为重要的作用。因此，一个新的学科名称——生殖内分泌学应运而生，并立即得到人们普遍的承认。在60年代，由于细胞和分子生物学的迅速发展，生命科学领域内传统学科之间的界线开始溶解，其结果使自然科学中各分支学科的大量信息和技术引进到生殖研究中，因而加速了它的发展，出现了一个新的学术领域——生殖生物学。生殖生物学把生殖内分泌学的研究推进到以前从未达到过的深度和广度。目前，激素（尤其是肽类激素）的定义已经发生了变化，被认为是细胞间的信使，经膜受体和细胞内介体，控制着靶细胞的基因表达。所以，过去激素调节概念就显得有一定的局限性，已不适应学科的发展。因此生殖生物学当前的研究方向多集中在细胞和分子的水平上，以便深入地阐述生殖活动的规律和机制，同时，由于生物技术的迅猛发展，进一步又推动了家畜繁殖业的提高和改善了人类生育控制的方法。

生殖生物学开放实验室概况

一、宗旨

1985年中国科学院建立了生殖生物学开放实验室(LRB),它作为一个基础研究单位,专门从事于生殖的研究。该实验室挂靠中国科学院动物研究所,行政管理由动物所负责。它的宗旨是办成一个国际性的生殖生物学研究中心,对世界上所有从事这一领域研究的学者开放。开放实验及其设备和研究经费(但不包括薪金)均可通过正式手续申请。

现在,开放实验室拥有4名教授、4名副教授和25—30名客座研究人员。

二、研究课题指南

开放实验室强调并鼓励生殖方面的基础研究和应用基础研究,优先考虑协作课题的研究。此外,本实验室也致力于发展在生育中具有重要调节作用的肽类激素和生物活性物质的生物工程研究,研究课题基本在下列指南范围之内:

1. 受精与着床机理的细胞学研究;
2. 生殖功能调节机理的离体研究;
3. 胚泡着床机理的研究;
4. 激素作用原理的研究(性类固醇和促性腺激素);
5. 神经肽和神经递质在生殖调节中的作用研究;
6. 促性腺激素基因的分离、结构分析和表达的研究;
7. 生殖免疫的研究。

三、研究进展(1986—1987):

近几年来,我们研究工作的重点集中于胚泡着床和妊娠的生理学和生物化学方面。胚泡着床是一个非常复杂的过程,它涉及到孕体和母体子宫之间的相互作用与协调。在我们的工作中,有一部分实验采用动物模型研究胚泡着床,另一部分工作则采用人的绒毛组织研究早期妊娠阶段胎盘功能的激素调节。实验结果简要总结如下:

1. 胚泡对子宫内膜代谢的影响

我们早期的实验表明,大鼠子宫内膜着床部位的^{3}H-尿嘧啶和^{3}H-亮氨酸的特异性放射活性显著地高于非着床部位。进一步的实验又发现,在着床部位和含卵的子宫角,其雌二醇受体和孕酮受体数量显著高于非着床部位和无胚泡子宫角。如把活的胚泡移植到假孕子宫内,则可促进子宫内膜蛋白质合成代谢速率的增加;移植灭活的胚泡(用酒精杀死)则无此影响。以上结果提示,胚泡释放的某些因子,参与了子宫内膜的代谢以及子宫细胞分化的调节,以完成子宫在接受孕体前的准备过程。

2. 兔胚泡特异肽的分离

为了证明着床前胚泡可释放某些活性因子,我们采用SDS-聚丙烯酰胺凝胶电泳,分析了孕、非孕和假孕的兔子宫冲洗液。在孕兔子宫冲洗液中分离并鉴定出三个特异性的多肽,分子

量分别为 3000，4500 和 6000，在非孕和假孕的兔子中没有发现相应的肽类。显然，这三种多肽来源于胚泡而不是子宫内膜。兔胚泡液经 HPLC 分析，也证明胚泡可合成和释放上述小肽。特别值得注意的是，妊娠 D1 和 D6 期的兔子，用高剂量 LH-RH-A（九肽）处理后，胚泡液中的小肽消失，说明 LH-RH 可抑制这些肽的合成。最近我们还发现，分子量为 4500 的小肽可抑制淋巴细胞的转化和前列腺素的产生。这一发现有重要的含义。进一步的工作正在进行之中。

3. 胚泡与子宫内膜的细胞识别

糖蛋白在细胞与细胞的识别中具有重要作用。为了探讨糖在着床中的作用，我们在小鼠子宫内注射了不同种类的糖，实验结果表明，棉子糖可有效地阻止着床（91%），糖元也有作用（60%），而半乳糖和甘露糖则没有影响。在实验中我们观察到二个有趣的结果：（1）阻断着床的效果与子宫内多核白细胞数量的增加呈相关性；（2）棉子糖注入到子宫角后，等电聚焦电泳中 pH 为 5.1 到 6.0 的几种蛋白质消失。以上工作还有待进一步的实验验证。

4. 胚盘绒毛中自我调节机理的研究

我们的实验表明，低浓度 LH-RH（或 GnRH）（10^{-9}M）刺激离体绒毛组织 hCG 的分泌，高浓度（10^{-6}M）时抑制 hCG 的释放，LH-RH 拮抗剂（D-Phe^{2},D-Trp^{6})-LH-RH 也使 hCG 释放减少。LH-RH 对孕酮的分泌也有相似类型的作用。在培液中加入 hCG 单克隆抗体可抑制孕酮的分泌。其他激素如雌二醇（E_2），睾酮（T），前列腺素（$PGF_{2\alpha}$）和促甲状腺素释放素（TRH）对 hCG 和孕酮的分泌均无影响。外源或内源性孕酮对 hCG 分泌也无作用，但低剂量 β- 内啡肽（10^{-8}—10^{-10}M）则可刺激离体 hCG 的合成和释放。

为了深入研究离体绒毛组织的激素调节，一个重要的问题首先是确定绒毛的细胞类型和各自合成特定激素的能力。绒毛中只有二类细胞即合体滋养层细胞和细胞滋养层细胞。虽然有大量文献报道有关这二类细胞各自的分泌能力，但至今没有得到一致的结论。我们采用改良的培养方法，分离到了高纯度的单一细胞群。实验结果表明，LH-RH 主要由细胞滋养层细胞所合成和释放，但它也能分泌孕酮及少量的 hCG。

5. 绒毛中神经肽和神经递质的研究

应用放免测定 HPLC 和 LCEC 等技术，我们在绒毛中发现了六种神经肽（甲硫氨酸 - 脑啡肽，亮氨酸 - 脑啡肽，β- 内啡肽，生长抑素，强啡肽和神经降压素）和三种神经递质（去甲肾上腺素，肾上腺素和 5- 羟色胺酸）。值得注意的是，神经降压素抗血清注入到子宫角能抑制着床，而生长抑素抗血清增加着床的胚胎数。

6. 生殖的比较生物学研究

（1）我们成功地完成大熊猫（*Ailuropoda melanoleucus*）精子获能和穿卵（仓鼠卵）的工作。

（2）豚鼠 Na^{+}-$K^{+}ATP_{ase}$ 活性和精子获能之间的关系。据我们所知，豚鼠精子质膜上 Na^{+}-$K^{+}ATP_{ase}$ 活性还没有人研究过，质膜用 0.05%DOC（脱氧胆酸盐）处理后，就可测到酶的活性。精子在获能培养液中孵育 48 小时后，Na^{+}-$K^{+}ATP_{ase}$ 活性，与对照组相比，下降到 35.6%；精子在含有 1μm 或 5μm quabain 的获能培液中孵育 10.5 小时，其顶体反应达 46.5 和 64.4%，而对照组只有 27.4%。结果提示，豚鼠 Na^{+}-$K^{+}ATP_{ase}$ 活性的下降加速了精子的获能。

（3）通过 HPLC 分析了文昌鱼体内的 LH-RH，结果表明，文昌鱼体内有两种 LH-RH，一种与哺乳动物的 LH-RH 相同，另一种与鲑鱼的 LH-RH 相同。

（4）肽类激素和其他医用化合物的生物技术研究在其他报告中另行说明。

四、实验室设备：

本开放实验室有先进的设备用于细胞和分子生物学研究。以下所列各项可供参考：

电子显微镜和超薄切片机；超速、高速及低速冷冻离心机 10 台；高压液相色谱 1 台；Leitz 及其他厂家的倒置相差显微镜 9 架；LKB 全自动 r- 计数器 1 台；LKB DNA 电泳仪 1 台；Bio-Rad 计算机电源电泳仪 3 台；LKB 多用途电泳仪 2 台；双光束紫外分光光度计 2 台；电化学检测仪 1 台；DNA 合成仪 1 台；荧光显微镜 1 架；显微镜和相差显微镜 5 架；解剖镜 5 架；ZF 细胞计数器 1 台；超声波破碎器 2 台；二氧化碳自动培养箱 9 个；冷冻浓缩干燥器 9 个；超净工作台 10 个；自动高压消毒锅 2 个；冷柜 3 个；活动冷库 1 个；录象设备 1 套；－80℃低温冰箱 1 台，－20℃低温冰箱 16 台，普通冰箱 18 台，制冰机 2 台；冷冻真空干燥器 2 台；AO- 冰冻切片机 1 台；电子天秤 8 架；以及大量小型设备。

五、学术委员会：

职能：1)从目前和长远的观点指导开放实验室的研究工作。2)评审年度报告；3)审批申请者及建议资助数额。

主席：张致一教授

成员：

郑丕留教授，中国农科院。

程治平教授，哈尔滨医科大学。

杨传任教授，北京农业大学。

肖碧莲教授，国家计划生育委员会科技研究所

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张致一教授，中科院生殖生物学开放实验室

曹咏清教授，中科院生殖生物学开放实验室。

庄临之副教授，中科院生殖生物学开放实验室。

六、开放实验室固定成员：

张致一教授，主任。

庄临之副教授，副主任。

曹咏清教授。

邹继超教授。

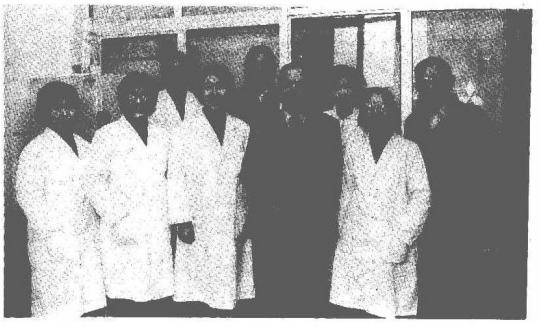
张崇理教授。

陈大元副教授。

沈孝宙副教授。

刘以训副教授。

王　红，助研，秘书。



生殖生物学开放研究实验室固定成员合影

Staff members of LRB.

七、申请程序：

申请报告：

申请人的申请报告，由学术委员会负责评审，评审会议于每年 2 月举行。申请时间没有明确的限制，但最好在早一年度 8 月 30 日前提出申请。申请报告必须与开放实验室的研究方向一致。

申请报告由以下几部分组成：

1. 设想：概述研究的内容和意义。
2. 发展趋势：陈述该研究领域的最新进展。
3. 实验设计：拟采用的方法和技术路线。
4. 特殊的要求：注明需要使用的仪器设备和特殊的化学试剂。
5. 背景材料：申请人的主要学历和工作简历。
6. 研究周期：完成该课题所需要的时间(不超过三年)。
7. 业务简历：包括近期发表的与本项目有关的文献目录。
8. 资助：申请通过之后，a)LRB 将提供研究经费；b)如果申请人需要住宿补助或自带研究经费，请在申请时注明。
9. 推荐意见：二名高级科研人员的推荐信。

通　　知：

申请报告经学术委员会评审通过后，申请人及申请人所在单位将收到通知，如果有必要可进行一次面谈。

研究课题的终止：

由于健康欠佳或其它原因，申请的课题不能按时完成，开放实验室主任有权终止其研究。

研究实验室或课题组介绍

一、肽类生化实验室

负责人：曹咏清

本室的主要研究目的是揭示发育中的胚胎与母体子宫之间复杂的内在联系。着床前的胚泡可产生活性因子，这一发现已为我们实验室和其它实验室研究人员所部分证实。这类因子可直接作用于子宫内膜局部区域。近年来，我们从着床前兔胚泡中分离到了三种生物活性肽，并进行了生化特性分析，证明都是小分子糖肽，分子量分别为 3000，4500 和 6000，它们具有重要的生理功能如促进子宫内膜蛋白质的合成，抑制前列腺素的产生。此外，它们还能抑制淋巴细胞的多核形转变。这一发现提示免疫系统可能参与妊娠。本室还与香港大学动物学系合作，从在体和离体小鼠胚泡中分离出胚泡肽，其结果也证实了在兔子中的发现。

我们对与着床有关的糖类也非常感兴趣。有关实验正在进行之中。

二、激素受体实验室

负责人：邹继超

本实验室主要从事于性激素与靶细胞（主要是雌性的生殖系统）受体的相互作用机理的研究。正在进行的工作可归纳为：1）雌激素及孕酮与它们受体亚单位特异性结合的特性；2）细胞内受体的产生和降解；3）膜上的性类固醇受体以及调节细胞活动的可能途径；4）影响激素和受体作用的因素；5）发展可用于基础研究和应用研究的新技术。

三、细胞培养和细胞工程实验室

负责人：庄临之

本实验室致力于阐述无血清细胞培养系统（包括下丘脑－垂体－性腺轴和胎盘绒毛）中激素作用的调节机理，研究细胞与细胞的相互作用，细胞功能的自分泌和旁分泌调节机理以及生殖器官细胞功能和形态分化的激素调节。目前，我们正在建立可用于筛选避孕药物和生物活性物质的高性能细胞模型。近几年我们也研究了妊娠早期胎盘绒毛激素自我调节的机理，探讨了β-内啡肽，生长因子、去甲肾上腺素和其他神经肽神经递质对绒毛的影响。此外，我们还与日本 Zeon 公司合作，通过细胞工程技术，进行麝香生产的研究。

四、生殖神经内分泌学实验室

负责人：张崇理

神经内分泌学作为一个独立的生物学科出现于 20 世纪中叶。其目的是揭示神经系统和内分泌系统的整合机理。过去该领域大部分工作集中于中枢神经系统而没有注意到胎盘绒毛。我们除了在生殖的内分泌调节方面做了许多工作外，近年来对胎盘绒毛的神经肽和神经递质开展了一系列的研究，以探讨它们的合成和释放部位及生理功能。本实验室工作的重点是 1）胎盘绒毛中神经肽和神经递质的功能，尤其是它们与激素释放之间的关系；2）脑内和胎盘绒毛组织中未知生物活性肽的纯化和特性分析；3）胎盘中神经肽和神经递质的免疫细胞化学定位；4）在低等脊索动物和脊椎动物中神经肽的结构和功能——神经肽进化的研究。

五、基因工程实验室

负责人：沈孝宙

本实验室利用重组 DNA 技术，研究促性腺激素及其有关活性肽类的基因表达，以及与生

殖过程有关的蛋白质的基因克隆，近几年，实验室致力于人生长激素基因在哺乳动物细胞中表达的研究。此外，还克隆了牛 MT 基因和酪蛋白基因，近期研究主要集中在以下几方面：1）hCG 在动物细胞工程中的高级表达；2）构建含酪蛋白启动区和靶基因的融合基因，以便在转基因动物的乳汁中特异表达外源基因产物；3）胚泡特异肽素的基因克隆；4）通过受精卵进行鱼类的基因转移。

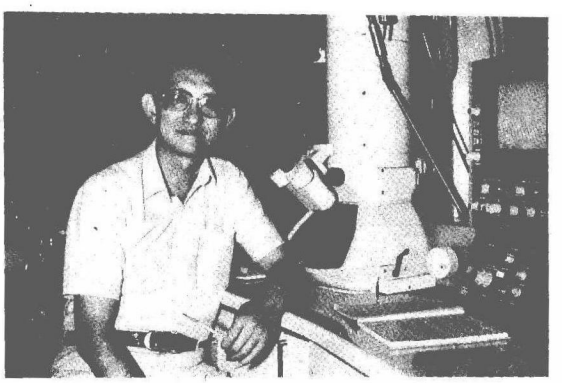
六、卵巢的细胞和分子生物学实验室

负责人：刘以训

本实验室的主要工作是 1）在卵巢功能的调节中，组织溶纤蛋白酶原激活因子（tPA）与它的抑制因子（PIA）之间的相互作用；2）排卵期间激素对 tPA 活性的影响；3）大鼠卵巢 tPA 基因的激素调节。据报道，在卵巢中已鉴定出二种 PA（tPA 和 uPA）和一种 PA 抑制因子。我们也观察到 tPA 和 uPA 存在于卵巢体细胞如颗粒细胞、内膜细胞和卵丘复合体。只有 tPA 与排卵有关。进一步的实验证实，卵巢膜细胞可产生一类 PA 抑制因子（PIA），它可部分或完全地抑制 PA 活性。非常有趣的是在卵细胞中也观察到了 PA 活性。所以，tPA 在卵细胞的成熟，卵丘细胞的扩散和排卵中都具有非常重要的作用。

七、受精与着床的细胞学实验室

负责人：陈大元



本实验室主要致力于阐明生殖内分泌腺细胞结构与生物功能的关系，正在进行的研究课题集中在以下几方面：1）受精过程中细胞骨架所参与的作用；2）濒危物种和家畜的体外受精及胚胎移植；3）在体内、外用超微技术和细胞化学技术研究胚泡发育和人胎盘绒毛在形态和功能上的分化。

RETROSPECT IN BRIEF ON THE PROGRESS OF REPRODUCTIVE RESEARCH:

Reproduction is an intrinsic element of life. The propagation of living organisms as well as the phylogenetic evolution would be made impossible without the very process of reproduction. Research on reproduction is of paramount importance from both societal and economical points of view, because it relates not only to population control, but also to the improvement in quantity and quality of animal protein production.

Reproductive biology, a science of comprehensive research on reproduction, made its first appearance somewhere in the middle part of 20 century as an independent discipline of life science. The rapidity of its progress has been followed more or less a similar track as the development of other branches of nature science, starting from descriptive study, succeeded by experimental investigation and finally becoming a multidisciplinary bio-science, with emphasis on molecular events of reproduction.

In retrospect, the reproductive physiology was probably the first one of the nature science dealing with reproduction and had made great strides in the early part of this century under the impetus of the introduction of the hormone concept into physiology by Starling. It progressed rapidily till 30's whilest a number of peptide hormones were extracted, purified and characterized. At about the same time, the chemical synthesis of sex steroids was also successfully accomplished. The new advance led the reproductive physiologists began to realize that it is the hormone which plays a major role in regulation of reproductive activities. A new terminology, reproductive endocrinology, was therefore coming into existence and immediately became widely accepted regardless whether intentional or not. In the late 60's as a consequence of the rapid advancement of cell and molecular biology, the traditional disciplinary boundaries were disolving among the fields of biological science and even between the science and technology. The situation permits and accelerates the infiltration of knowledge and techniques from other branches of nature science into reproductive research and thus results in the emergence of a new discipline, the reproductive biology, which has extended the horizon of reproductive endocrinology to a level that has never been achieved before. The hormone (peptide hormones in particular) is now considered as an intercellular messenger which via membrane receptor and intracellular mediator, controls the gene expression of the target cell. The old concept of hormonal regulation of reproduction was therefore felt rather restrictive and could not keep abreast of the new developments and hence, the reproductive biology endeavors now to elicit more deeply into the underlying mechanism that controls the reproductive activities at the cellular and molecular levels. Meanwhile, the fast progress of biotechnology further advances the reproductive study toward improvement of livestock production and betterment in methods of human contraception.

AN OVERVIEW OF THE LABORATORY OF REPRODUCTIVE BIOLOGY:

A. INTRODUCTORY DESCRIPTION:

The Laboratory of Reproductive Biology (LRB) was established in 1985 by the Chinese Academy of Sciences as a basic research unit engaged in the study of reproduction. It is affiliated to the Institute of Zoology with provision for maintenance. The LRB is intented to be an international center for reproductive research open to people all over the world who are interested in this field. Laboratory space with facilities and funds for running costs, but not salaries, can be made available through a formal application.

The LRB at present comprises four professors and four associate professors. In addition, there are about 25 to 30 visiting scientists .

B. RESEARCH GUIDE-LINES:

Both basic research and application-oriented studies on reproduction are emphasized and encouraged by the Laboratory. High priority is given to intramural collaborative research. The LRB also endeavors to develop biotechnological study on peptide hormones or biological active compounds that are of significance in regulation of fertility.

1. Cytological studies on fertilization and implantation.
2. In vitro studies on the regulatory mechanism of reproductive function.
3. Mechanism of embryo implantation.
4. The principle of hormone action(sex steroids and gonadotropins).
5. The role of neuropeptides and neurotransmitters in regulation of reproduction.
6. Gene isolation,structure analysis and expression of gonadotropins.
7. Immuno-reproductive research.

C. RESEARCH ACTIVITIES(1986-1987):

In recent years, our major research activity was centered on biochemical and physiological aspects of embryo implantation and pregnancy. Embryo implantation is a highly sophisticated process of interaction and coordination between the conceptus and maternal uterus. Our experiments were performed partly with animal model for investigation of implantation and partly with human trophoblastic tissue for study of hormonal regulation of plancetal function at early gestation stage. The results are briefly summarized as follows:

1. Local effects of blastocyst on metabolism of uterine endometrium:

As we have priviously shown that the specific radioactivities of ^{3}H-uridine and ^{3}H-leucine of the implantation sites of the rat endometrium were significantly higher than those of the non-implantation segments. This local metabolic difference was further verified by the finding that the amount of estradiol and progesterone receptors was greater in the implantation site and the ova-

containing horns of the uterus than those in the non-implantation region and the non ova-containing horns. Furthermore, the metabolic rate of protein synthesis in the uterine endometrium was striking affected by the presence of transferred live blastocysts, while the implanted inactivated blastocysts (killed by alcohol) were found to be ineffective. The above experimental results implicate that some factors released by the blastocysts may take part in regulation of the uterine metabolism and cellular differentiation which could be a prerequisite for the uterine readiness to receive the coming conceptus.

2. Specific peptides isolated from the rabbit blastocysts:

In order to identify the existance of the active principles or factors released by the pre-implantation blastocysts, the uterine flushings of pregnant, non-pregnant and pseudopregnant rabbits were analysed by SDS-polyacrylamide gel electrophoresis. Three specific peptides with molecular weights of 3,000, 4, 500 and 6, 000 were isolated and identified. No corresponding peptides were detected in non-pregnant and pseudopregnant uterine flushings. Obviously they were derived exclusively from the blastocysts other than the uterine endometrium. Analysis of the rabbit blastocyst fluid by HPLC confirms the above finding, indicating once again that some specific peptides are synthesized and released by the blastocysts. It is of particular interest to note that treatment of rabbits with high dose of LH-RH-A (a nona-peptide) from D1 to D6 of pregnancy led early disappearance of these unidentified peptides from the blastocyst fluid, suggesting that LH-RH inhibited the synthesis of the peptides. Recent finding demonstrates that one of these peptides (MW 4, 500) inhibits lymphocyte blastogenesis and prostaglandin secretion ($PGF_{2\alpha}$). The result presented above is of paramount importance. and further experimental work is in progress.

3. Cell-to-cell recognition between blastocyst and uterine endometrium:

Since glycoprotein plays an important role in cell to cell recognition, a few preliminary experiments have been carried out by intra-uterine administration of a variety of sugars in mice with the purpose to explore the possible involvement of sugars in implantation. The results indicate that raffinose is highly effective(91%) in prevention of implantation. Glycogen is also effective(60%); while galactose and mannose are ineffective. There are two interesting findings that may be of significance: 1) there is a correlation between the efficacy of inhibitable action and the increase in number of polymorphonuclear leukocytes of the uterus; 2) raffinose when introduced into the uterine horn leads to the disappearance of a few isoelectric proteins between pH 5. 1 and 6. 0. The significance of this observation remains to be studied.

4. A self-regulatory mechanism exists in placental trophoblast:

Experiments have demonstrated that the LH-RH (or GnRH) of the trophoblast is stimulatory in nature at low dosage level (10^{-9}M) and inhibitable at higher concentrations (10^{-6}M) on the production of hCG in vitro. The LH-RH antagonist (D-Phe^2, D-Trp^6)-LH-RH, on the other hand, decreases the hCG secretion. A similar paradoxical effect as also observed in experiments dealing with LH-RH influence on progesterone production. Addition of monoclonal hCG antibody into the culture inhibits progesterone secretion. Other hormones, such as E2, T, $PGF_{2\alpha}$ and TRH were without effect. Neither exogeneous nor endogeneous progesterone exerts any influence on hCG

secretion. However, β-EP at dosages between 10^{-10} and 10^{-8}M stimulates the synthesis and release of hCG in vitro.

For further analysis of hormonal regulation by the trophoblastic tissue in vitro, it is important to define the cell type which is capable of synthesis the particular type of hormone. As we all know there are only two kinds of cells present in trophoblast tissue, the syncytiotrophoblast and the cytotrophoblast cells. Although there were a great number of reports in literature dealing with their respective secretory capacity, no definite conclusion as yet has been agreed upon. Using a modified culturing method, we were able to separate these two types of cell and a relative pure cell culture has been obtained. As a result of this study, it was demonstrated convincingly that LH-RH is synthesized and released exclusively by the cytotrophoblast cells, which secrets also progesterone and, to a lesser extent, minute amount of hCG.

5. Neuropeptides and neurotransmitters in trophoblast:

Experiments have been conducted with first trimester trophoblast tissue by RIA, HPLC and LCEC techniques. It was found that there are 6 neuropeptides, Met-Enk, Leu-Enk, Dynorphin, β-endorphin, somatostatin, and neurotensin as well as 3 neurotransmitters, NE, E, and 5-HTP being present in the trophoblast. It is of interest to note that the antiserum of neurotensin when injected into the uterine horn inhibited implantation. On the other hand, intra-uterine administration of somatostatin anti-serum increased the number of implanted embryos.

6. Works on comparative biology of reproduction:

1) We have sucessfully accomplished the work on sperm capacitation and egg penetration (hamster) in the giant panda (Ailluropoda melanoleucus).

2) The relationship between Na^+,K^+-ATPase activity and sperm capacitation in guineapig. As far as we are aware, the $Na^+,K^\pm$ ATPase activity in the plasmalemma of guinea pig sperm has never been detected before. By treating the sperm plasmalemma with 0.05% DOC (deoxycholate), it was found that the enzyme activity could be measured. After incubation of spermatozoa in the capacitation medium for 8 hours, the Na^+,K^+-ATPase activity decreased to 35.6% as compared with that of the unincubated group. The spermatozoa incubated for 10.5 hrs. in the capacitation medium containing 1 or 5 μM quabain showed 46.5% and 64.4% acrosome reaction respectively in contrast to the 27.4% acrosome reaction of the control. It was suggested that the decrease in Na^+,K^+-ATPase activity enhances sperm capacitation in guinea pig.

3) LH-RH of Amphioxus was investigated by HPLC. The result showed two separate peaks, one of which resembles very much in nature to the mammalian peptide and the other one, to the salmon, a kind of bony fish.

4) Biotechnological researches on peptide hormones as well as other medical compounds will be presented in a separate report.

D. LABORATORY EQUIPMENT:

The LRB is well equipped for research in cell and molecular biology. The items are listed below for reference:

Electron-microscope and ultra-thin microtones (1); Centrifuges, ultrahigh, high and low speed

refrigerated centrifuges (10)*; High performance liquid chromatograph (1); Leitz and other types of inverted phase contrast microscopes (9); r-Counter (1); LKB macrophor electrophoresis unit (1); Bio-Rad computer controlled electrophoresis (3); LKB multiphor electrophoresis (2); Double-beam ultraviolet spectrophotometer (2); Electron-chemical detector (1); DNA synthesizer (1); Flurescence microscope (1); Microscope and phase-contrast microscopes (5); Disecting microscopes (5); ZF cell counter (1); Ultrasonic cell breakers (2); CO_2 automatic incubators (9); Refrigerated concentration trap(1); BioGARD hoods(10); Automatic autoclave(2); Cold-LAB(4); Movable cold room(1); Video set and TV (1); Deep freezer, −80 C (1), −20 C (16); Refrigerators (18); Ice makers (2); Lyophilizers(2); AO-cryomicrotone(1); elctron-balances (8); and a great number of smaller items.

* Indicates number of pieces.

E. SCIENTIFIC ADVISORY COMMITTEE:

Functions: 1. To provide guidance to the Director on the research activities of the Laboratory from a near and a long range viewpoint.

2. To evaluate the annual report.

3. To review and comment on research proposal submitted by individual scientist with suggestion for funding.

Chairperson: Zhang Zhi-yi (Chih-Ye Chang).

Members: Zheng Pei-liu, Professor, Chinese Academy of Agriculture Sciences.

Cheng Zhi-ping, Professor, Harbin Medical University.

Yang Chuan-ren, Professor, Beijing University of Agriculture.

Xiao Bi-lian Xiao, professor, National Research Institute for Family Planning.

Xue She-pu, Professor, Institute of Basic Medical Sciences.

Shi Qi-xian, Associate professor, Zhe-jiang Academy of Medicine.

Gu Zhi-ping, Associate professor, Shanghai Institute of Materia Medica.

Zhang You-duan, professor, Institute of Biology, Shanghai.

Zhang Zhi-yi, Professor, Laboratory of Reproductive Biology.

Cao Yong-qing, Professor, Laboratory of Reproductive Biology.

Zhuang Lin-zhi, Associate professor, Laboratory of Reproductive Biology.