生殖生物学开放研究实验室

论文选集

Laboratory of Reproductive Biology

Selected Papers

1985–1990

中国科学院动物研究所

Institute of Zoology, Academia Sinica

中国 北京
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（1914–1990）

第七届全国政协委员，中国科学院生物学部委员、代理主任，中国动物学会理事长，生殖生物学会理事长，国际比较内分泌学会理事，国际生物学联合会中国委员会顾问，亚太地区比较内分泌学会荣誉会员，中国科学院动物研究所研究员、学术委员会主任、生殖生物学开放研究实验室主任，中国科学院发育生物学研究所学术委员会主任，《动物学报》编委会编委，我国著名动物学家、胚胎学家、生殖生物学家张致一先生。

前　言

今天是中国科学院生物学部委员、代理主任，我国著名动物学家、胚胎学家、生殖生物学家，中国科学院动物研究所生殖生物学开放研究实验室主任，我国生殖生物学奠基人之一张致一教授逝世一周年。张致一教授知识渊博，学术造诣深，在我国胚胎学和生殖生物学等领域有杰出成就，为我国科学事业的发展贡献了毕生精力。为纪念这位科学伟人，我们特收集了1985-1990年间在张致一教授直接领导下生殖生物学开放研究实验室在国内外核心刊物上发表的论文，汇编成册，以志纪念。

编　者

1991年10月8日

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，它与自然科学其它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。

张孝攸

一九八五年

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RESEARCHES ON REPRODUCTIVE BIOLOGY ——A REVIEW *

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This is a brief review centered mainly on reproductive researches carried out by the author and his associates at the Department of Endocrinology. Part of the works not affiliated with this laboratory but relevant closely to this subject is also included. Attempt was made to arrange the text chronologically (at least under each topic) in order to illustrate the trend of research and the historic progress. Literatures from other laboratories are not cited in this review.

I. Sex Determination and Differentiation in Amphibia and Aves

1. *Amphibians*

At the early stage of sex differentiation in Amphibia the undifferentiated gonads consist of a cortex and a medulla, both of which elaborate some kind of factors or inductors essential for orientation of gonadal sex differentiation. Under the dominance of the cortex, the gonads will be destined to develop into an ovary. If the medulla becomes dominant, a testis is formed. When the germ cells are located in the cortex, they will be determinated to differentiate into eggs. If they migrate and settle in the medulla, they will develop into sperms.

The sex of Xenopus can be reversed at young stage. The genotype of Xenopus in male is homogametic (ZZ) and the female, heterogametic (ZW). By administering minute amount of estrogen into the ambient water of the Xenopus tadpoles, all of the young larvae were feminized. The androgen, on the other hand, was ineffective and the sex proportion among the Xenopus remained unaltered. This is true also for other animals with heterogametic female constitution. When the sex-reversed adult female (ZZ) was mated to a normal male (ZZ), their offsprings were 100% males[1,2]. Parabiosis between a male and a female at very young stage or grafting of a differentiating testicular gonad (ZZ) to the position adjacent the undifferentiated ovary(ZW), the ovary could be induced to undergo sex reversal[2]. Upon removal of the grafted testis at proper age, the host ovary evently became a fertile testis, a ZW male. If such sex-reversed ZW male was bred to a normal ZW female, 1 / 4 of her offsprings were genetic males and 3 / 4, females. By further breeding experiments, fertile WW could be obtained, which produced 100% female offsprings when mated to a normal male.

* Thanks are due to Prof. Y-X Liu who did excellent work in editing the manuscript.

In Xenopus, the undifferentiated gonads are a segmented structure appearing as a pair of elongated bodies. It has been demonstrated that there is a critical stage of sex differentiation which occurs at Stage 26 and lasts about 2 days. By careful selection of hormonal treatment at the critical time, one could pinpoint the sex reversal of the gonads along the antero-posterior axis, indicating the existence of an antero-posterior polarity during sex differentiation[3].

Analysis of sex differentiation in Amphibians by treating the undifferentiated animals often fails to reveal the chronological changes of sex transformation. Examination of sex glands in a small number of experimentally produced hermaphrodites is, in most cases, insufficient to furnish the desired information. We have performed an experiment by starting the hormonal treatments in the sexually differentiated animals at the late developmental stages, and hence able to observe more closely the direct action of hormone on the gonadal transformation process[4]. All the animals used in this investigation were males derived from a sex-reversed female(ZZ). One group consisted of 10 newly metamorphized juvenile animals treated with estradiol and another 10 animals at the age 30 days after metamorphosis received the same treatment as the first group. The predominant character produced in the first group is the strong stimulation and prominant proliferation of the ovarian sac. This furnishes unequivocal evidence that sex reversal in the female direction is in progress. Oogonia develop in the gonad, but cortical differentiation was not observed in most cases. In the second experimental series, the animals received hormone treatment 30 days after metamorphosis and continued for another 60 days, showed rapid development of the ovarian cortex, growth of oogonia and formation of oocytes. Melanophores appeared in the gonad, a faithful sign of feminization characteristic of this species. Oviducts persisted in both experimental groups. The results of this study revealed two undiscovered facts concerning hormone action and sex differentiation. (1) There is a difference between the cortex and the medulla in response to steroid sex hormone at different developmental ages. The medullary part responsed most readily to estradiol immediately after metamorphosis. It has also demonstrated that estradiol is acting directly on the cortex and the medulla, and is not mediated through an indirect route. There was no evidence to show that the hormone inhibited the development of the medulla, which secondarily permitted the cortex element to grow and differentiate as proposed before by other authors. (2) The great stimulation and development of the cortex in the second group of experiments seemed to deserve special consideration. Since at the beginning of the hormonal treatment, cortex has already disappeared, the presence of a newly formed cortex would certainly have to come from the tissue other than the original cortex element. The results have not been reported previously and are of significance, for it serves as another example of pluripotentiality of the post embryonic tissues.

The genotype of Bufo(toad) is similar to that of Xenopus. So their response to sex steroids is the same. However, the Bufo is probably the only animal possessing a rudimentary ovarian structure present in both sexes throughout their whole life. In female it is indistinguisable from the mature ovary and in male, it is located at the uper part of the testis. Removal of the testis will result in growth of this organ into a fertile ovary. It is rather surprising to find that the near-by testis does not exert any masculinization effect on the sex differentiation of this rudimentary ovary, even though the Bufo have the same sex genotype as Xenopus. Prolonged treatment of toad larvae with estrogen and androgen did not influence the sex differentiation of the Bidder's organ. Recent finding has shown that the sex steroid receptors are present in this structure. The lack of medullary element of Bidder's organ may provide a reasonable explanation for this intriguing problem in nature[5,6].

In Rana the female is homogametic (XX) and the male, heterogametic(XY). Both undifferentiated gonads of genetic male and female react with sex steroids positively, i.e., androgen masculinizes the ovary and estrogen feminizes the testis[7]. However, estrogen at high concentration induces adrenal hyperplasia and masculinization of the ovary. By treating

the intact and hypo-physectomized young larvae with high dosage of estradiol, we have demonstrated that the estrogen-induced hyperplasia of adrenal is restricted to animals still in possession of their hypophysis; in other words, adrenal hyperplasia is an indirect reaction mediated by the hypophysis[8]. This conclusion is sustained by the fact that adrenals of hypophysectomized larvae are easily restored to normal size and activity by administration of ACTH. It is, therefore, evident that in the frog sex reversal and adrenal hyperplasia are independent reactions to estradiol administration. However, there remains at least a possibility that estradiol if administered in high dosages, might become a source of production of a hormone with androgenic properties. The conditions under which adrenals may become androgenic are obviously not realized by the experimental setup. Adrenal cortical hormones such as desoxycorticosterone and cortisone are androgenic. The hyperplasia adrenals induced by high dose of estradiol were found to be able to secret steroids as shown by paper chromatograph but the chemical structure has never been analysed[9]. Since estrogen does not induce adrenal hyperplasia in hypophysectomized animals, it seems that the estrogen stimulates directly the release and production of ACTH by the hypophysis.

In conclusion, the information so far accumulated about genetic determination of sex in Amphibia suggests a possible relationship between female monogametic constitution(XX) and positive masculinization response. It has been established that in Rana the female sex is monogametic. On the other hand, in Ambystoma, Pleurodles, Xenopus and Bufo, the females are digametic(ZW). Ovaries seem to respond to administered androgens if they are of homogametic constitution. This result is significant in view of the fact that heterogametic ovaries do transform into testes, namely under the condition of testicular implantation. It must be concluded that the androgenic steroids are not identical with the effective agents under the condition of latter experiments.

2. *Aves*

In order to establish the hypothesis proposed in the foregoing paragraph that a possible relationship exists between female monogametic constitution and positive masculinization response, it is necessary to extend the experiments to different taxonomic classes of vertebrate, especially to those of the warmblooded animals. The problem is of interest not only for its theoretical consideration but also for practical application.

Chicken would be the best choice for experimental study. The sex genotype of the male chicken is homogametic (ZZ) and the female, heterogametic (ZO). White leghorn eggs were used in these study. Two types of hormonal treatments have been performed: (1) injection of estrogen, diethylstilbestrol (DES), and testosterone into the incubating eggs; (2) immertion of un-incubated or incubating eggs into the hormonal solution[10]. The results demonstrate clearly that estradiol when injected into the incubating eggs before the onset of sex differentiation will result in feminization of the genetic males. Ovaries and oviducts developed normally in most of the cases. Surpressingly, it was found that ovaries of the sex-reversed young animals were unstable and they eventually, a few months after hatching, returned to their original sex. Prolonged hormonal treatments were ineffective in maintaining such acquired sexuality. In study of the process of secondary sex inversion in sex-reversed male chicks, it is observed that the right rudimentary gonad possesses a strong hereditary factor for testicular differentiation, and initiates always testicular development in advance of the left ovary. This is believed to be responsible for the instability of the femaleness in such experimental animals.

Contrary to the reports of other investigators, the present study demonstrated that the feminization effect of DES was incomplete. Microscopical study of the gonads revealed that both cortex and medulla were present. Continuous hormone injection after hatching could

sustain the ovotestis for a long time, but the cortical component of the gonad would gradually disappear soon after the discontinuation of hormonal treatments. The development of the oviducts in such animals was completely inhibited. It is of interest to note that estradiol in immersion experiments failed to modify the development and differentiation of the embryonic gonads. Testosterone, on the other hand, was without obvious effect on gonadal differentiation. The results show that the external genitalis of the embryonic chick responses to sex hormones more readily than the gonad. The former gives positive response to probably all steroid sex hormones applied, while the latter, only to certain types of hormone and hence with a higher degree of specificity. The results obtained in this investigation furnish unequivocal proof to show that the embryonic sex inductor is not identical with sex hormone produced by the mature sex glands.

II. Studies on Comparative Endocrinology

1. *Developmental endocrinology*

It is well known that the hypothalamus plays an important role in regulation of endocrine activity via the release of peptide hormones. The techniques employed in the early '60s involved mainly with electric lesions, stimulation or implantation of various chemicals or hormones. These methods contributed much to the understanding of the hypothalamic functions. Complete hypothalectomy, removal of the whole hypothalamus, in adult as well as in embryos had never been successful acomplished until 1957 when the senior author reported for first time the success in extirpation of the hypothalamic preprimordium in anuran embryo[11]. The operation was performed at the open neurula stage. A rectangular piece including the presumptive hypothalamus tissue togather with the underlying hypoblast was removed. The developing tadpoles showed light coloration due to the contraction of melanophores, which since the classical work of B. M. Allen has been considered as a characteristic consequence of the ablation of the primordium of the buccal hypophysis. On the other hand, the development of the hindlegs was not impaired. Tadpoles developed normally just short of the extrusion of the fore-arms. The climax of metamorphosis was inhibited[12]. Microscopical examination on operated animals showed that the infundibulum was missing and the hypophysis laid free in the infundibular canal of the basilar cartilage, some distance below the brain. The results demonstrated that the differentiation of the intermediate lobe depended on induction from the hypothalamus, while that of the anterior lobe was not or to a minor degree only. The delay of climax metamorphosis suggested that ordinarily a sudden increase of the output of TSH may be required during this critical stage. It was fairly clear that the full functional activity of the hypophysis depended on hypothalamic stimulation[13].

During the time of '50s, there was a long disputed question whether ACTH (adrenocorticotrophic hormone) and MSH (melanophore stimulating hormone) were identical hormone or separate entities. The significance of this implication was fully appreciated by Waring and Ketterer (1953, Nature) who put it in this way: "this claim has obvious academic interest, but it also raises an issue of importance for those concerned with research technique and clinical practice, because the assay for melanophore-stimulating properties is simple and reliable, while the currently used ACTH assays are not". According to one group of the investigators, the two hormones were identical. Their arguments were based mainly on the following observations: (1) there was a correlation between the rise of both ascorbic acid depletion and MSH activities in the blood of human patients suffering from Cushing's syndrome, Addison's disease and pregnancy, etc., (2) the chromatographic separation of the two substances from partially regenerated corticotropin A_1, one possessing both ACTH and

MSH activities and one inactive in both tests, provided evidence that both activities were intrinsic in one molecule; (3) melanophore response could be obtained by 0.01 μg of commercial ACTH preparation and this high activity could not be due to contamination. On the other hand, the opposite view was held by some whose claims were based more on physio-chemical criteria rather than on pure clinical observations. The evidences were: (1) potentiation of MSH and inactivation of ACTH by alkali-heat treatment; (2) partial separation of MSH and ACTH activities by using a discontinuous pH gradient on cellulose; (3) differential migration of activities during zone electrophoresis on filter paper; (4) differential concentration of ACTH and MSH activities in the adenohypophysis and intermediate lobe of the pituitary gland. Per unit of wet weight, the adrenal ascorbic acid depleting activity of the adenohypophysis was at least twice that of the intermediate lobes, whereas the melanophore stimulating activity of the intermediate lobe was approximately 60 times as great as that of adenohypophysis. Both groups of the investigators insisted on their own views and it was difficult to reach a general agreement in the absence of an unequivocal proof. The failure to reach a consolidating agreement may perhaps be explained either by the lack of a common criterion used to assay their materials being almost impossible to measure simultaneously the ascorbic acid depleting and MSH activities in the same individual, or by lack of knowledge concerning the nature of pigmentary change in human beings. Neurohumoral control of pigmentary changes in amphibian has been demonstrated in this experiment. However, any claim that there is similar mechanism involved in the cold-blooded animals and mammals is speculative and should be cautious.

The results of this study has presented strong evidence to support the theory that ACTH and MSH are separate hormones[14]. That a tadpole which lacks an intermediate lobe but possesses a free and functional adenohypophysis shows constant contraction of melanophores, is a good indication that the adrenocorticotrophic hormone is not responsible for the expansion of melanophores. Later biochemical analysis provides convincing evidence that they are indeed separate chemical entities, but share a common fragment of amino acid sequence.

Removal of hypophysis and hypothalamus as well as the thyroid gland in developing amphibian larvae not only affects the growth and function of the reproductive organs, but also influences the enzymatic activity of non-reproductive organs. The relationship between hormone and alkaline phosphatase in developing animals had been studied by many investigators. Most of the works were performed in intact young animals. Thus the endogenous hormonal interference was not excluded. By using hypothalectomized, hypophysectomized and thyrodectomized Rana larvae,the alkaline phosphatase activity in intestine was studied[15]. The results showed that removal of hypophysis and thyroid significantly inhibited enzyme activity, but not in hypothalectomized animals. Injection of ACTH and cortisol in such operated larvae, on the other hand, increased the activity of alkaline phosphatase. High concentration of estradiol, which was shown previously causing adrenal hyperplasia in intact tadpoles, was without effect. In conclusion we are justified to say that pituitary-adrenal axis plays an important role in regulation of enzyme activity and the thyroid hormone is probably also involved. It is suggested that alkaline phosphatase may be used as a criterion for assaying of endocrine disorders.

2. *Mechanism of ovulation*

Ovulation is a highly complicated process of reproductive activity. Expelling of a mature ovum with its follicular cells from the ovary is a prerequisite for successful fertilization. Failure of ovulation will lead to infertility. The theory that ovulation is regulated and coordinated by an array of hormonal interplay has been well verified and widely accepted. However, the underlying mechanism relevant particularly to the cellular and molecular events of ovulation

is far from fully apprehending. The works presented herewith is a brief summary of our results published elsewhere.

Ovulation in fishes Induction of ovulation or spawning of fish was initiated from economical point of view. China has not only a vast inland water resourse but also a long history of fresh-water pisciculture. The four well-known Chinese farm fishes are recognized in the world by their large size, superior meat quality and fast growth rate. Unfortunately, they do not spawn naturally in the pond, so that the production was limited. A LH-RH nonapeptide synthesized by Shanghai Institute of Biochemistry was available in the early part of '70s and was found to be a highly effective ovulating agent for induction of spawning of the farm fishes. The results of this works will be referred to again later. At time being, the discussion is restricted only to some of the basic researches.

The grass carp, just like the case in other farm fishes, do not spawn naturally in confined water, but they could be easily induced to do so by treatment with LH-RH or its nonapeptide analog. Histochemical studies indicated that in teleost, just like in mammalian species, the functional changes of the pituitary and the ovary are directly or indirectly under the control of the hypothalamic releasing hormone[116]. It was demonstrated that the medium-lobe (adenohypophysis of fish) of the grass carp's pituitary has only one type of gonadotroph which contains two kinds of granules differing in size and stain reaction. The gonadotrophs showed extensive activity of hormone synthesis and release following LH-RH treatment. This was evidenced by the progressing decrease in number of the small granules, and increase in number and size of the giant heterogenous granules accompanied by the appearance of cytoplasmic vacuolation. The small granules are likely to be the LH secreting granules and the giant ones, the FSH producing granules. Owing to the rapid elevation of the hypophyseal LH, the ovary was greatly activated. This was confirmed by the finding that the enzyme activities of G-6-Pase, AKP, ACP and 3β-OH-SDH in the follicular cells of the ovary were greatly stimulated after hormonal treatment. The result suggests that the steroid hormone plays an important mediating role in the process of gonadotropin action.

The histochemical studies were followed up by electron-microscopic investigation for the purpose of revealing more details at the subcellular level[117]. In untreated control fish, three types of secreting granules could be differentiated in the gonadotroph, namely the small granules, the globular granules and the giant heterogenous granules. In the experimental group, the following cellular changes were observed 2 or 6 hours after hormonal injection: (1) a fusion of the gonadotrophs; (2) an enlargement of the endoplasmic reticulum cisternae; and (3) a decrease in number of both small and globular granules. While, on the other hand, the giant granules continued to increase in size, owing probably to the aggregation of the individual granules. After spawning, gonadotrophs were loaded with endoplasmic reticulum cisternae. The small granules became sparse, but the number of the giant heterogenous granules kept increasing. The experiment demonstrated again that the small granules are likely to be the LH secreting granules while the giant ones are probably the FSH containing granules. In conclusion, it seems reasonable to state that in fish, at least in grass carp, there is only one type of gonadotroph which synthesizes and releases two kinds of gonadotropins, LH and FSH.

The current accepted concept bearing on the mechanism of action of the peptide hormone is generally explained on the hypothesis of hormone receptor. According to this theory, there are specific hormone receptors located on the surface of the plasma membrane of various target cells and the activation of such receptors by hormone initiates the generation of cAMP which mediates a series of physiological responses. However, recent finding suggests that a different mechanism of hormone action may be involved, which is based upon the experimental evidence showing that the peptide hormone is capable of being internalized into the target cells[118]. Nevertheless direct evidence was scanty. By means of electron-microscopic autoradiography, we demonstrated the presence of labeled hormone intracellularly in the

gonadotrophs of the fish hypophysis. It was further shown that the labeled hormone was internalized not only into the cytoplasm of the gonadotrophs, but also into the nucleus, apparently via the nuclear pores. The fact is of interest because it is probably the first direct proof showing that the peptide hormone (LH-RH) may act directly on the genome to regulate gene expression. Competitive binding between labeled and the cold hormones showed a 50% inhibition of radio-activity of the pituitary gland. This means that the electron dense particles represents truly the active hormone, not the free iodine.

Ovulation in amphibian In an early report on estrogen-induced adrenal hyperplasia we had referred to the possibility that adrenal hormones might play a supporting role in the induction of ovulation. The suggestion derived from the observation that high levels of estrogen, such as this which ordinarily precedes ovulation, affects not only the gonadotropic output of the hypophysis but also the release of ACTH This concept was sustained later by an in vitro-experiment concerning cortisone effect on ovulation in frog[19]. The entire ovarian lobes were ligated with silk thread, resected, and placed in Petri dishes with 20 ml buffered amphibian Ringer's solution. The results demonstrated that the cortisone was, indeed, exhibiting a facilitating role in ovulation. However, the cortisone was effective only if it acted on follicles that had been brought close to the ovulation threshold by other hormonal factors, among which the LH-progesterone sequence may be assured to be the most important. Since in vitro experiments has shown that hypophyseal hormones in large dosage may independently produce ovulation, cortisone did not appear to be an indispensable factor. Cortisone was also demonstrated in fish having a similar supporting role in ovulation.

The above works were further extended to another species of amphibian, the Bufo[20-22]. The ovarian fragments in these experiments were suspended by cotton thread in vials containing 20 ml of amphibian Ringer's solution buffered at pH 7.3. In order to completely eliminate the interference of endogenous hormone on the responsiveness of the ovarian tissue, hypophysectomy was performed 3 to 6 days prior to the start of each experiment. A sub-minimal effective dose(SMED) of pituitary extract was obtained and determind by in vitro method for each batch of pituitary preparations. Comparative studies on ovulatory potency of various steroids were first conducted in vitro with normal ovarian fragments obtained from freshly killed non-hypophysectomized animals. The results showed that the order of potency among them are desoxycorticosterone 〉 progesterone or testosterone 〉 androsterone 〉 dehydroepiandrosterone 〉 adrenosterone 〉 cortisol 〉 cortisone. Analysis of the role of hormonal action was performed entirely with ovarian fragments of hypophysectomized toads. The results demonstrated that: (1) there were four kinds of steroids (desoxycorticosterone, testosterone, progesterone and cortisone) which were capable of inducing ovulation without the assistance of pituitary factor; while the other four hormones (androsterone, dehydroepiandrosterone, adrenosterone and cortisol) did not possess the ovulating capacity in the absence of pituitary factor. Species specificity in response to hormonal treatment was noted in case with cortisone which was shown in a previous report being incapable of inducing complete ovulation in Rana. Regarding to pituitary hormones, contrary to the report of Burgers and Li (1960), GH, LH, hPL, and ACTH, when tested on ovarian fragments of hypophysectomized animals, were unable to induce ovulation in vitro except in cases where they were used in combination with minute amount of pituitary extract. The results obtained here indicated that some of the pituitary hormones as stated above were not ovulatory in nature when they were used alone. However, a supporting role in ovulation was clearly seen as they were used in combination with a sub-minimal ovulating dose of pituitary extract. It was remarkable to note that there existed a time difference between the two different kinds of hormones in reacting with the ovarian fragments required for the initiation of ovulation. A latent period of one hour was observed in experiment with pituitary extract, while only a brief 10. minute treatment was found to be enough for induction of

ovulation by progesterone. A latent period like this would suggest that a special mechanism of molecular interaction may be involved. Experiments were also conducted to analyze the nature of hormone action by: (1) subjecting the ovarian fragment first to pituitary suspension and then transferring into progesterone solution; (2) similar in method as above, but used purified GH and hPL instead of pituitary extract. The results showed that enhancement of ovulation was demonstrated in the first series of experiments while no effect was found in the second group. The data presented here suggested that sensitization of ovarian follicles by pituitary factor possibly render them more competent to other ovulating hormones, and the pituitary gonadotropin , FSH, was considered to be the primary factor responsible for such an action. Estradiol, did not enhance progesterone induced ovulation in vitro but it did exert an inhibitory effect on pituitary induced ovulation. Evidence implicate that a short period of contact (1 to 2 hours) between estradiol and ovarian tissue was sufficient to produce a profounded inhibitory effect on the responsiveness of the ovarian follicles to the subsequent pituitary action. It demonstrated clearly that estradiol acted directly on the ovarian tissue with a consequence of diminishing sensitization to ovulating hormones. For the purpose to clarify the question: "Does the in vitro ovulation represent a normal physiological process? In other words, are the ovulated eggs fertile?" Eggs released from progesterone and desoxycorticosterone induced ovarian fragments were tested for fertility. The fertilized eggs cleaved and developed normally in every respect.

The follow-up work (unpublished) gave further information helpful in understanding of ovulation process. The experimental results demonstrated that the ovulatory effect of LH could be blocked by the addition of indomethasine to the culture medium, implicating the possibility of the involvement of prostaglandins (PG_s) in ovulation. This was proved to be true by the finding that $PGF_{2\alpha}$was highly effective in the induction of ovulation. It was of particular interest to note that db-cAMP significantly inhibited ovulation in vitro. In the presence of db-cAMP the ovulatory potencies of both LH and progesterone were greatly reduced. In another experiment it has shown that LH stimulated the generation of cAMP by the ovarian fragments, reaching a peak 60 minutes after the treatment and decreasing thereafter. On the other hand, progesterone effectively inhibited the production of $_{ovine}$cAMP. The results presented above suggest a pituitary-prostaglandin-progesterone axis plays an important role in the process of ovulation, obviously via a route bypassing the second messenger. Further elucidation remains to be investigated.

The hypothesis was once again verified by a different approach. The rest potential of the Bufo ova during ovulation under various experimental conditions was measured by a microelectro-physiological method[23]. It was shown that the pituitary extract stimulated a rapid elevation of the rest potential which decreased gradually after 30 minutes. Contrary to LH, Progesterone lowered the potential in a time-dependent manner. If the two graphs were superimposed, a very interest picture was revealed. There were apparently two phases existed during the process of ovulation. A pituitary or LH phase was represented by the high level of rest potential while a progestin phase was depicted by the lower profile of potential. As far as we know, this interest phenomenon has not been reported before.

Ovulation in mammal Ovulation in mammals has drawn great attention not only by reproductive biologists but also by embrydologists. Theories concerning the mechanism of ovulation are varied but no one was accepted without skeptic. It is shown in recent years that plasminogen may be involved. Plasminogen activators convert plasminogen into plasmin, which is a protease that cleaves fibrin into fibrin fragments. Two types of plasminogen activators (tPA and uPA) have been identified in ovarian granulosa cells and they are considered to be involved in ovulation. Recent study has shown that the denuded oocytes contain tPA but not uPA and the ovarian somatic cells contain both[24]. However, only tPA activity is regulated by the gonadotropins and reaches a maximum just prior to ovulation[25]. Previously