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Meiotic Error of the Superovulated Oocytes in Mouse

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= Abstract =Chromosomes were studied in DBA/2N mouse oocytes after superovulation. Twenty female mice were divided into 2 groups. Group I animals were induced to superovulate by injections of 5 IU PMSG and 5 IU hCG, and group II animals were injected with 10 IU PMSG and 10 IU hCG. Two cases of chiasma were found among the oocytes in group II. The rest of oocytes showed normal chromosomes in metaphase II. These results demonstrated that PMSG and hCG-induced superovulation correlated with chiasma formation.

Key words: ***Meiosis, Superovulation, Oocyte, Mouse, Chiasma formation***

INTRODUCTION

As in most other mammals, maturation of the mouse oocyte occurs in the follicle immediately before ovulation, and the oocyte remains at metaphase II in the uterine tube until fertilization. If any defective events are present in some oocytes during meiosis, these oocytes and their descendents may carry chromosomal anomalies. Although virtually most of investigations about chromosomal analysis of the eggs have employed superovulation techniques, there were insufficient cytogenetic evidences to demonstrate that gonadotropins influence the chromosome constitution of the embryos. The difficulty of obtaining high quality chromosome preparations could be one reason why only very few investigations have been carried out.

Meanwhile several investigators reported that incidences of chromosomally abnormal embryos have been associated with PMSG (pregnant mare's serum gonadotropin) and hCG (human chorionic gonadotropin)-induced superovulation in mammals (Vickers 1969; Donahue 1972; Kaufman 1972, 1973; Fujimoto *et al.* 1974). Takagi and Sasaki (1976) proposed that the increase in digynic triploidy after superovulation would be due to suppression of either the first or the second polar body. In addition, since an improved method for preparing chromosomes from mouse embryos have been developed recently (Dyban 1983) it seemed worthwhile to investigate the effect of administration of gonadotropins on meiotic division of the mouse embryos.

MATERIALS AND METHODS

Animals

Inbred DBA/2N female mice, 7 to 13 weeks old were obtained from Laboratory of Animal Physiology and Genetics, Department of Biology, Chonbuk National University. In order to examine the differences between the two gonadotropin dosages, twenty mice were divided into 2 groups of 10 females. Group I animals were induced to superovulate by an intraperitoneal injections of 5 IU PMSG (Sigma Chemical Co.) followed 5 IU hCG (Sigma Chemical Co.), and 10 IU of PMSG and hCG were given to Group II animals. hCG were injected 47 hr after administration of PMSG. Unfertilized oocytes were recovered 21 to 23 hr after the hCG injection and according to the methods described by Jeong *et al.* (1984).

Chromosome preparations

Chromosome preparations were made by modification of the descriptions of Tarkowski (1966) and Dyban (1983). The oocytes were washed out from the uterine tubes with Ham's F-10 medium. The cumulus cells were removed with hyaluronidase (1 mg/ml, Sigma Chemical Co.). After 5 to 10 minutes, the oocytes were washed with fresh medium. They were subsequently placed in 2 ml of cold 0.5% potassium chloride in a precooled watchglass and left in the 4°C refrigerator for 2 hours. With the help of a mouth-controlled pipette, a microdrop of hypotonic solution together with

several oocytes is placed in the middle of a grease-free slide under a dissecting microscope. The diameter of a drop did not exceed 5 mm. After expelled the oocytes on the slide, the excess solution around the oocytes was removed with a mouth-controlled pipette. Fixative (3 parts of methanol, 1 part of glacial acetic acid) was drawn into a pipette and expelled over the oocytes before the hypotonic solution was evaporated. The slides with fixed oocytes marked with oil pen and were dried overnight on a warming table. For chromosome staining 2% Giemsa at pH 6.8 was used. After staining for 15 minutes, the slides were washed under tap water and were dried at room temperature. The analysis was focused principally on the morphology of the chromosomes in oocytes ovulated.

RESULTS

From 20 animals of two groups, 450 oocytes were collected and chromosome analyses were made on 112 oocytes or 24.9% of the eggs collected. Most of the remaining preparations of the eggs were unsuitable for analysis because chromosomes were either inadequately spread or lost from broken oocytes. Totals of 50 and 62 oocytes were analysed from group I and group II respectively. Two cases of chiasma were found among the 62 oocytes of group II. These results indicated that PMSG or/and hCG affected ovulations of the immatured oocytes. All the chromosomes of the oocytes in group I showed metaphase II. Two cases of chiasma were found in different slides, and one of them showed more earlier stage than the other case. Extended bivalents and loop shaped chromosomes at early diakinesis were observed in the chromosomes of an oocyte (Fig. 1). While in the other case there were more contracted bivalents and chiasma terminalizations at late diakinesis (Fig. 2). Some bivalents took on the appearance of cross yet, the others showed chiasma terminalization with the homologues joined to each other at their terminal ends. Only parts of the number of chromosomes were able to find in the slides. These were assumed to be due to an artificial loss. The morphology of the chromosomes did not lend itself to precise identification of missing or extra chromosomes. But the majority of oocytes in group II and all of the oocytes in group I showed haploid number of metaphase II chromosomes.

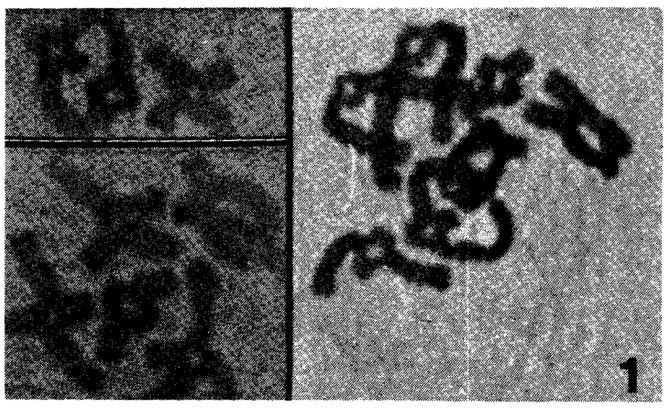

Fig. 1. Photomicrograph of chromosomes in early diakinesis showing high chiasma frequency. Reproduced at 1000x.

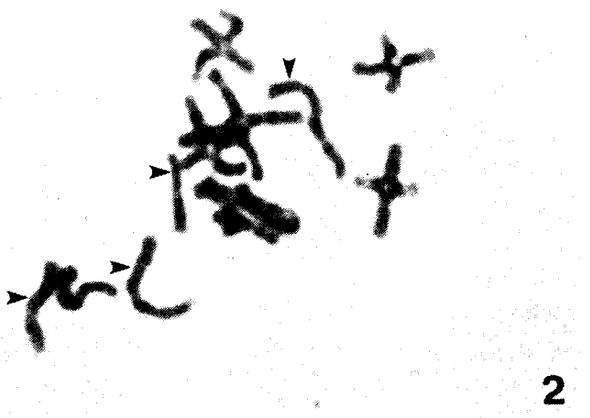

Fig. 2. Photomicrograph of chromosomes in late diakinesis showing chiasma rotation and chiasma terminalization (arrow). Reproduced at 1000x.

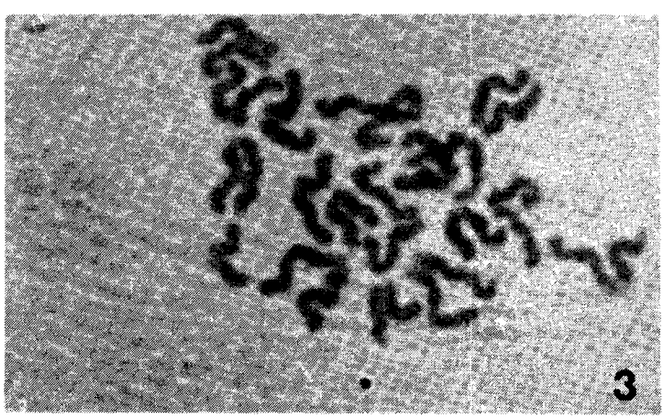

Fig. 3. Photomicrograph of second meiotic metaphase chromosomes. Reproduced at 1000x.

DISCUSSION

In the chromosome preparations of our experiments, we were able to find 2 cases of chiasma in the oocytes ovulated by injection of 10 IU gonadotropins. Two cases showed a slightly different stages of the diakinesis each other.

The mechanism involved in the proccess of oocyte maturation following the endogenous release or exogenous administration of LH is not well known at present. But interesting aspect of our results is the ovulation of primary oocytes. Normally mammalian oocytes resume meiosis I immediately prior to ovulation, and enter metaphase II in a short period. Foote and Thibault (1969) rendered this phenomenon by proposing the breakdown of the stalk of cells connecting the oocyte with the granulosa cells lining the follicle in pig. Oocytes resume maturation at that time. This observation also suggested that LH may act by some physiological process to cause this isolation of the oocyte. Then, what is the cause of first meiotic error in the superovulated oocytes by injection of 10 IU gonadotropins? Sugawara and Mikamo (1983) suggested that the inadequate tubulin polymerization by some agents, which causes incomplete formation of spindle microtubules may also be a direct cause of the increased incidence of meiotic chromosome nondisjunction in the oocytes of aged mothers. In addition, metaphase I begins some 3-4 hours after an injection of hCG into mice primed with PMSG (Edwards and Gates 1959). Many oocytes, however, can fail to respond to the hormone and over-stimulation of the follicles can lead to premature luteinization (Henderson and Edwards 1968). And Takagi and Sasaki (1976) suggested that superovulation could suppress the polar body formation. By these observations and suggestions, it would appear that superovulation could induce a genetically premature luteinization of the intrafollicular oocytes, and some of the oocytes might fail to end meiosis I by inhibition of tubulin polymerization. Thus some primary oocytes or oocytes with suppressed polar body may also be ovulated. Suppression of first polar body formation is recognized as a possible cause of triploidy and may be one cause of fetal death in human.

On the other hand, the mammalian primary oocyte is known to be capable of resuming process of meiosis when removed from the ovarian follicle and incubated in vitro (Chang 1955). And several works on the maturation of mouse oocytes in vitro have been reported (Edwards 1962, 1965; Donahue 1968; Jagiello 1969). In the present study, even though all of the oocytes had stayed in the uterine tubes for 9 hours after ovulation until uterine tubes were flushed, two oocytes did not complete the meiosis I in the oviductal fluid. Therefore it would appear that a few premature oocytes ovulated by injection of gonadotropins could not resume maturation.

We found only 2 cases of chiasma in the present study, but our preliminary results demonstrated that gonadotropins can stimulate oocyte growth and ovulation. But a certain dosage of gonadotropins cannot induce genetic maturation in some of the stimulated oocytes, on the other hand, inhibit process of meiosis. Actually we couldn't find cases of chiasma into oocytes of group I. Donahue (1972) also found accidently metaphase I oocytes in his analysis of the first cleavage division in superovulated mouse embryos. Induction of superovulation by PMSG and hCG injections has been widely adopted for obtaining a large number of eggs or conceptus from laboratory animals. The results of the present study indicate the need to consider the possible genetic effects of superovulation on oocyte maturation.

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= 국문초록 =

과배란된 생쥐난자의 염색체에서 발견된 감수분열이상

충북대학교 의과대학 해부학교실 및 서울대학교 의과대학 해부학교실*

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Inbred DBA/2N 생쥐에 PMSG와 hCG를 각각 5 IU씩 주사한 군과 10 IU씩 주사한 두가지의 실험군을 설정하고, 과배란을 유도하여 배란된 난자에서 염색체의 형태를 조사해 본 결과, 10 IU를 주사하여 과배란된 난자 2예에서 아직 미성숙 상태임을 의미하는, 다수의 chiasma를 형성하고 있는 염색체를 발견하였다. 이 사실은 과배란 유도시에 미성숙 상태의 난자가 배란된다는 세포유전학적 한 증거가 될 수 있다.