

Cyclic AMP Concentrations in Follicular Fluids: Relationship to Outcomes of In Vitro Fertilization Cycles¹

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= Abstract = Follicular fluids and their matched oocytes were obtained from 16 follicles of 7 women who conceived after in vitro fertilization (IVF), and 49 follicles of 17 women who failed to conceive after the procedure. Follicular development was induced with a combination of follicle-stimulating hormone and human menopausal gonadotropin.

There was no significant difference in follicular cyclic AMP concentrations between preovulatory and immature oocytes. However, follicles of preovulatory oocytes obtained from women who conceived after IVF contained lower cyclic AMP concentrations than those from women who failed to conceive after the procedure. Follicles of cleaved oocytes from pregnant women contained lower cyclic AMP concentrations than those of cleaved oocytes from nonpregnant women or all uncleaved oocytes.

These results suggest that lower cyclic AMP concentrations in follicular fluids are associated with successful pregnancies in IVF cycles.

Key words: *Cyclic AMP, Follicular fluid, In vitro fertilization*

INTRODUCTION

The concept of oocyte maturation is important in human in vitro fertilization (IVF) because oocytes are aspirated from follicles at an unknown and variable time before ovulation would normally occur. The exposure of oocytes to spermatozoa before the completion of maturation may result in fertilization failure, retarded and abnormal embryo growth and reduced embryo viability.

The luteinizing hormone (LH) surge is responsible for resumption of meiosis in the oocyte, a critical step in oocyte maturation. However, the mechanism by which LH induces the resumption of meiosis is not well known. While it is well accepted that cyclic AMP mediates LH action in the ovary (Marsh 1976), its role in the regulation of oocyte

maturation is not clear. Both inhibitory and stimulatory effects of this nucleotide on the oocyte maturation were reported (Dekel and Beers 1978; Tsafiri *et al.* 1972).

Therefore, this study was undertaken to evaluate the cyclic AMP level in follicular fluid in relation to outcomes of IVF cycles.

MATERIALS AND METHODS

1. Subjects

Sixty-five follicles with their attendant oocytes were aspirated from 24 women who underwent an IVF procedure at Seoul National University Hospital. Sixteen samples of follicular fluid were obtained from 7 women who conceived after IVF, and 49 samples from 17 women who failed to conceive after the procedure (Table 1).

2. Ovarian stimulation

Follicular development was induced with a combination of follicle stimulating hormone (FSH) and human menopausal gonadotropin (hMG), as described by Muasher *et al.* (1985). The only mod-

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Table 1. Maturity and cleavage rate of aspirated oocytes

Pregnancy outcome	Oocyte maturity	Total No.	No. cleaved
Pregnant women (n= 7)	Preovulatory	13	11(85)
	Immature	3	1(33)
	Total	16	12(75)
Nonpregnant women (n=17)	Preovulatory	43	34(79)
	Immature	6	3(50)
	Total	49	37(75)

Figures in parenthesis are percentages

ification was that hMG was administered at 6:30 PM. Two ampules of FSH (Metrodin, Serono Laboratories, Inc.), containing 75 IU of FSH and <1 IU of luteinizing hormone in each ampule, and two ampules of hMG (Pergonal, Serono Laboratories, Inc.), containing 75 IU of FSH and 75 IU of LH in each ampule, were administered intramuscularly on days 3 and 4 of the cycle. Stimulation was continued with hMG alone from cycle day 5 onward, usually in the dose of two ampules daily. hMG administration was continued until serum estradiol value reached 400 pg/ml or until an estrogen-mediated shift in the vaginal maturation index and cervical mucus scores were noted for 3 consecutive days. Human chorionic gonadotropin (hCG), 10,000 IU, were administered when the leading follicle reached a diameter of 16 mm. Laparoscopy with follicular aspiration was performed 34 to 36 hours after hCG administration. Recovered oocytes were graded as preovulatory or immature, as described by Veeck *et al.* (1983). The fluid from each

individual follicle was centrifuged at 300 × g for 10 minutes, and the cell-free sample was frozen for further analysis.

3. In vitro fertilization procedure

Insemination was performed with sperm concentrations of 5 × 10⁵/ml after 6-8 hours of preincubation for preovulatory oocytes or 26-34 hours for immature oocytes in Ham's F-10 medium supplemented with 7.5% human fetal cord serum. Ham's F-10 medium supplemented with 15% fetal cord serum was used for embryo culture. Oocytes were examined daily to identify cleavage. At 2 or 3 days after insemination, embryos were transferred to the uterus.

4. Radioimmunoassay

All follicular fluids were measured for cyclic AMP concentrations by radioimmunoassay using a commercial kit (Immuno Nuclear Corp.), and the procedure suggested by the manufacturer. Intra- and interassay coefficients of variation was 7.4% and 11.9%, respectively. Serum estradiol concentration was measured by radioimmunoassay using a estradiol-ter kit (Serono Laboratories). Intra- and interassay coefficient of variation was 5.2% and 7.5%, respectively.

5. Statistical analysis

Statistical analysis was carried out between groups using Student's t-test.

RESULTS

Cyclic AMP concentrations in follicular fluids were significantly lower in women who conceived after IVF than those who failed to conceive after the procedure (16.0 ± 3.0 pmol/ml vs 37.8 ± 4.1 pmol/ml, p < 0.005, Table 2).

Overall, follicles of immature and preovulatory

Table 2. Relationship among cyclic AMP levels (Mean ± SEM) in follicular fluid, pregnancy outcome, and the maturity of oocytes

Pregnancy outcome	Oocyte Maturity	Cyclic AMP (pmol/l)
Pregnant	Preovulatory (n=13)	15.5 ± 2.8*
	Immature (n= 3)	18.0 ± 11.9
	Total (n=16)	16.1 ± 3.0**
Nonpregnant	Preovulatory (n=43)	36.8 ± 4.6*
	Immature (n= 6)	45.0 ± 7.3
	Total (n=49)	37.8 ± 4.1**

Figures in parenthesis are numbers of oocytes

*P < 0.01 t=2.50, **P < 0.005 t=2.09

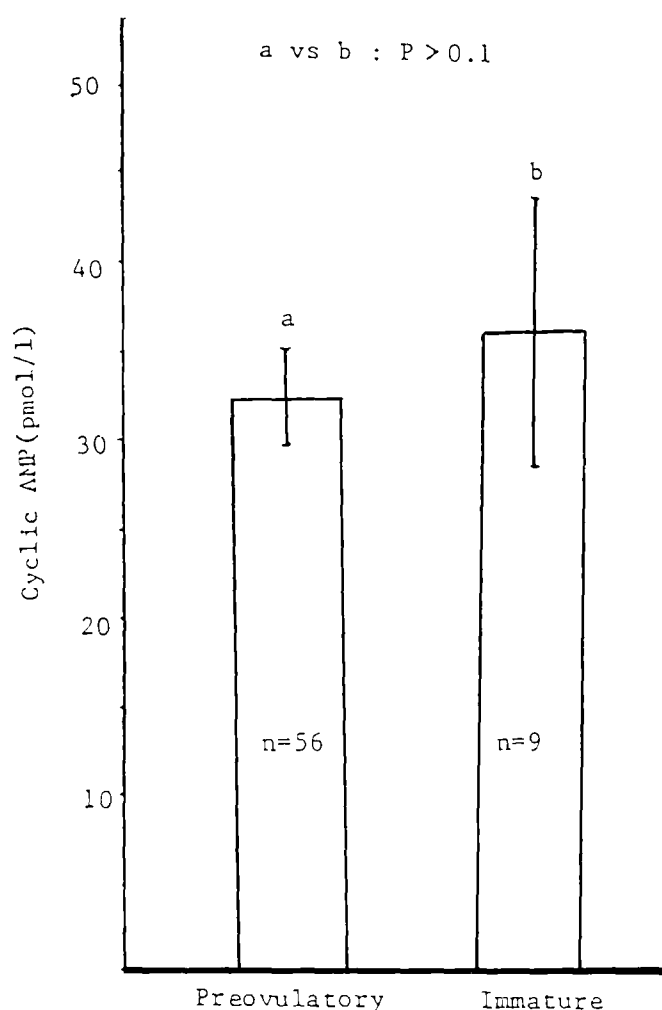


Fig. 1. Cyclic AMP levels (Mean \pm SEM) in follicular fluid according to the oocyte maturity.

oocytes did not differ in cyclic AMP concentration (31.9 ± 3.8 pmol/ml vs 36.0 ± 7.4 pmol/ml, $p > 0.1$, Fig. 1). However, follicles of preovulatory oocytes obtained from pregnant women contained lower cyclic AMP concentrations than those from nonpregnant women (15.5 ± 2.8 pmol/ml vs 36.8 ± 4.6 pmol/ml, $p < 0.01$, Table 2).

There was no significant difference in follicular

cyclic AMP levels between cleaved and uncleaved oocytes (29.1 ± 2.8 pmol/ml vs 36.8 ± 4.6 pmol/ml, $0.05 < p < 0.1$, Fig. 2). However, follicles of cleaved oocytes from pregnant women contained lower cyclic AMP concentrations than those of cleaved oocytes from nonpregnant women or all uncleaved oocytes (14.2 ± 3.9 pmol/ml vs 33.9 ± 3.4 pmol/ml $p < 0.005$ 14.2 ± 3.9 pmol/ml vs 42.8 ± 10.5 pmol/ml, $p < 0.05$, Table 3).

DISCUSSION

The LH surge is responsible for resumption of meiosis in the oocyte (Thibault 1977; Moor 1977), a critical step in oocyte maturation. However, the mechanism by which LH induces the resumption of meiosis is not well understood. Although it is well accepted that cyclic AMP mediates LH action in the ovary (Tsafiriri *et al.* 1972; Hillensjö 1976; Marsh 1976), the exact role of cyclic AMP in the regulation of oocyte maturation remains controversial, and conflicting results have been reported.

Many investigators suggested that cyclic AMP may be involved in the intrafollicular inhibition of oocyte maturation (Cho *et al.* 1974; Wasserman *et al.* 1976; Magnusson and Hillensjö, 1977; Dekel and Beers 1978; Dekel and Beers, 1980). In contrast to the inhibitory effects, there are also experimental evidences suggesting that cyclic AMP and its derivatives may actually have stimulatory effects on meiosis under certain conditions (Tsafiriri *et al.* 1972; Hillensjö *et al.* 1978). Preovulatory follicles respond to gonadotropin administration with increased cyclic AMP formation (Marsh *et al.* 1973; Nilsson *et al.* 1974). And the increase in follicular cyclic AMP concentrations after hCG administration was followed by rapid decline of elevated levels, namely, the desensitization. Recently, some investigators (Ekholm *et al.* 1984; Yoshimura and Wallach

Table 3. Relationship among pregnancy outcome, cyclic AMP levels (Mean \pm SEM) in follicular fluid, and the cleavage of oocytes

Pregnancy outcome	Cleavage	Cyclic AMP (pmol/l)
Pregnant	Cleaved (n=12)	$14.2 \pm 3.9^{*,**}$
	Uncleaved (n= 4)	21.3 ± 7.6
Nonpregnant	Cleaved (n=37)	$33.9 \pm 3.4^*$
	Uncleaved (n=12)	49.9 ± 13.3
Total	Uncleaved (n=16)	$42.8 \pm 10.5^{**}$

Figures in parenthesis are numbers of oocytes

* $P < 0.005$ $t=3.22$, ** $P < 0.05$ $t=2.36$

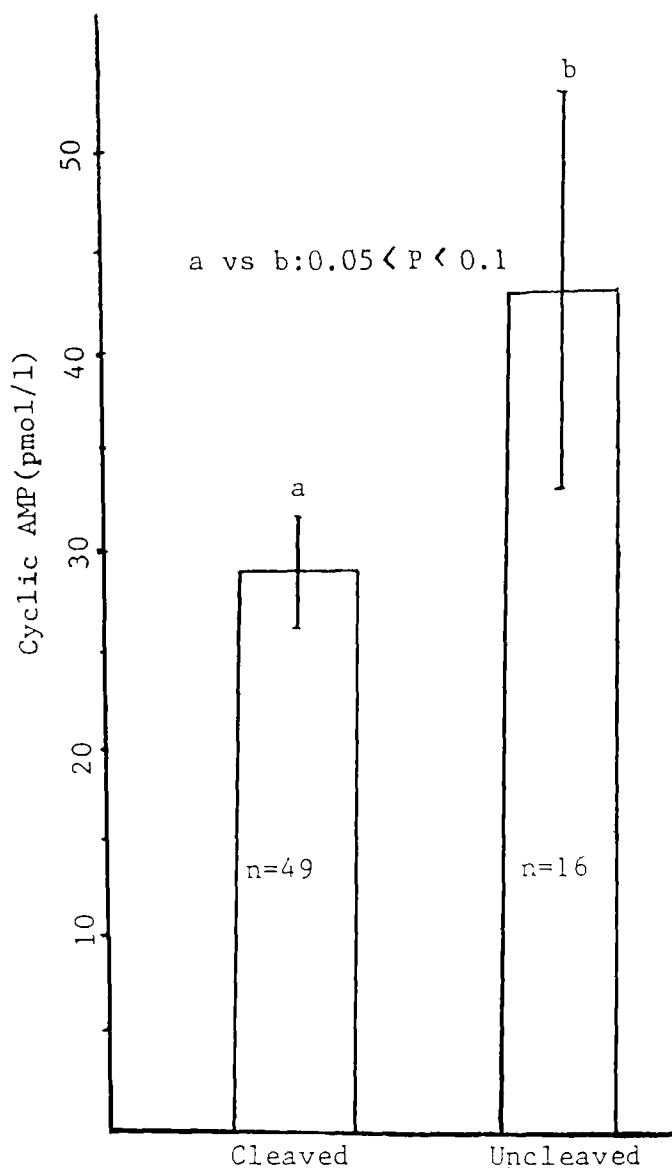


Fig. 2. Cyclic AMP levels (Mean ± SEM) in follicular fluid according to the cleavage of oocyte.

1987) suggested that a transient increase in follicular cyclic AMP, such as the LH-induced cyclic AMP surge, trigger maturation, but continuous elevation of cyclic AMP levels exert an inhibitory effect on oocyte maturation.

In our study, oocytes from pregnant women after IVF were derived from follicles which contained lower cyclic AMP concentrations than those from nonpregnant women. This finding, which is consistent with a report by Tarlatzis *et al.* (1984), suggests that lower follicular cyclic AMP levels are associated with more optimal development of follicles and oocytes. Lower follicular cyclic AMP levels may indicate that desensitization has occurred before aspiration, and that follicles and oocytes were optimally stimulated.

In this study we examined the relationship be-

tween the follicular cyclic AMP levels and the oocyte maturity according to oocyte-cumulus-corona complex appearance. We observed no difference in follicular cyclic AMP levels between preovulatory and immature oocytes. This finding is not likely to contradict the suggestion that lower cyclic AMP levels in follicular fluids are associated with more mature oocytes. No difference observed in follicular cyclic AMP levels between preovulatory and immature oocytes may be explained by the inaccuracy of morphologic criteria in the assessment of oocyte maturation. At aspiration the oocyte is surrounded by an investment of cells, the corona and the cumulus oophorus, which make direct visualization of the oocyte difficult; it is frequently impossible to tell whether the first polar body has actually been extruded. And, although the appearance of those investing cells is related to oocyte maturity, the evaluation is subjective and varies according to the experience of the observer. And asynchrony between individual cumulus-corona cell complex and oocyte maturation may occur in hyperstimulated cycles, as reported by Laufer *et al.* (1984). In addition to nuclear maturation, oocyte maturation also involves cytoplasmic changes resulting in a defense against polyspermy, established by the migration of granules from the cytoplasm to the cortex of the ooplasm. Cytoplasmic maturation is essential for normal fertilization and early embryonic development.

Our observation that preovulatory or cleaved oocytes from pregnant women were derived from follicles containing lower cyclic AMP levels further suggests that lower cyclic AMP levels in follicular fluids may be required to develop more optimal oocytes leading to a conception.

In conclusion, lower levels of cyclic AMP in follicular fluids are associated with successful pregnancies in in vitro fertilization cycles.

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= 국문초록 =

체외수정주기에 있어서 난포액내 cyclic AMP치에 관한 연구

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체외수정 및 배아의 자궁내이식 프로그램에 있어 그 성공율을 높이기 위해서 여러개의 성숙된 난자를 얻는 것이 매우 중요하다. 현재로서 난자의 성숙도는 일반적으로 난자-난구의 형태에 따라 분류하고 있으나 그 판정에 있어서 주관적이고 정확성이 결여되어 있다.

난자의 성숙은 LH surge에 의하여 유발되며 cyclic AMP가 LH의 2차적인 messenger라는 점에서 cyclic AMP가 난자성숙에 관여하리라는 가능성이 제기되어 왔다. 그러나 cyclic AMP와 난자성숙과의 관계에 대하여 현재까지의 여러 보고들이 일치되어 있지 못하다. 이에 저자들은 체외수정주기 24례(임신주기 7례 및 비임신주기 17례)에 있어서 흡인된 난포액 65례의 cyclic AMP치를 측정하여, 난포액내 cyclic AMP치와 난자의 성숙도, 난할여부, 배아의 자궁내이식 후 임신여부와와의 관계에 대하여 조사하였다.

1. 임신된 주기에 있어서 난포액내 cyclic AMP치 (16.1 ± 3.0 pmol/l: mean \pm SEM)는 임신이 안된 주기 (37.8 ± 4.1 pmol/l)보다 유의하게 낮은 값을 보였다 ($P < 0.005$).
2. 난자-난구 형태에 따른 판정에 의한 성숙된 난자 (31.9 ± 3.8 pmol/l)와 미성숙난자 (36.0 ± 7.4 pmol/l) 사이에 난포액내 cyclic AMP치는 유의한 차이가 없었다 ($P > 0.1$).
3. 임신된 주기에 있어 난할이 일어난 난자의 경우 (14.2 ± 4.0 pmol/l) 난할이 일어나지 않거나 (42.8 ± 10.5 pmol/l) 혹은 난할이 일어났으나 임신이 안된 경우 (33.9 ± 3.4 pmol/l)보다 난포액내 cyclic AMP치가 유의하게 낮은 값을 보였다 ($P < 0.05$).

이상으로 미루어보아 난포액내 cyclic AMP치는 성공적인 임신으로 이끄는 최적 난자 상태와 연관될 수 있다는 것을 시사하여 준다.