Investigation of adenosine metabolites in human follicular fluid: A pilot study correlating oocyte maturation and development

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Purines have been suggested to arrest meiotic development in vivo as well as in vitro. In this prospective study, we determined whether purines obtained from the follicular fluid (FF) of patients artificially stimulated for IVF-ET cycles could serve as markers of oocyte maturity and fertilization. Multiple FFfrom four patients were analyzed for the relative amounts of hypoxanthine, adenine, adenosine, cAMP, inosine and guanine by HPLC method. A possible correlation were not observed between the purines and oocyte maturity and fertilization rate, with the exception of guanine whose levels appeared to be higher in the more mature oocytes. [Turk J Med Res 1995, 13(3): 94-96]

Key Words: Follicle, Oocyte, Purines

The mitotic division of oogonia is completed in the majority of species the time right before or shortly after birth and the oogonia need to enter prophase of meiosis in order to progress to oocytes. Oocyte maturation is arrested at diplotene stage of the first meiotic division until just before ovulation. However this inhibition is lifted if the oocyte is removed from its follicular environment. It has been proposed that a specific factor within the follicle maintains the meiotic arrest of the oocytes (1). A substance capable of maintaining this arrest the so-called oocyte maturation inhibitor (OMI) is found in porcine follicular fluid (FF) and its effect can be potentiated in vitro by cyclic adenosine monophosphate (cAMP) (2).

Purines exert their effects by inhibitng the cAMP phosphodiesterase activity and increasing cAMP levels. If the oocyte responds to signals of the cumulus mediated by cAMP, decreasing the flow of purines and thus cAMP may uncouple a link between these cell types lead to the resumption of meiosis. Removal of the oocytes by aspiration during the in vitro fertilization setting could mimic this effect. The fact that a reduction in intraoocyte levels of cAMP is associated with resumption of meiosis is consistent with this idea (3). Other purines have also been implicated in the maintainance of meiotic inhibition and it has been shown that adenosine and/or hypoxanthine to inhibit oocyte maturation in vitro (4).

In the present study we assessed the purine levels in the follicular fluids obtained from stimulated cycles in humans and correlate these levels with the oocyte maturation and fertilization rates. To determine whether the levels of any purines in the human follicular fluid purines could function as a marker for oocyte capable of being fertilized in IVF cycles.

MATERIALS AND METHODS

Twenty-six follicular fluid samples were obtained from 4 patients randomly chosen from (age±SD: 33.8±1.5 years, range 32-35) undergoing in-vitro fertilization program between December 1991-September 1992. All the patients had tubal factor. None had male factor based on the WHO criteria (5). Treatment was started on day 21 of the menstrual cycle by daily subcutaneous injection of leuprolide. On day 2 of the subsequent menstruation, daily treatment with metrodin and human menopausal gonadotropin at an individualized dose ranging from 1 to 3 ampules per day was started and follicular development was monitored by assaying serum estradiol and ultrasound scanning of the ovaries. Adjustment in dosage of gonadotropins were based on the results of estradiol (E2) levels and ultrasound examinations. Human chorionic gonadotropin (hCG) was administered (10000 IU) intramuscularly when the lead follicle attained a mean

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Table 1. Induction cycle characteristics of the patients studied

	Number of Follicles	Number of Oocytes	Maximum Estradiol (pg/mi)	Day of hCG Injection	Fertilization Rate (%)
Patient 1	15	11	850	9	77.3
Patient 2	17	12	958	10	75.0
Patient 3	20	13	987	9	85.2
Patient 4	14	9	878	10	78.0

diameter of 18 mm and E2 were>200 pg/ml per follicle. The ultrasound guided oocyte retrieval procedures were performed 34-36 hours after the injection of hCG.

Right after the aspiration oocyte maturity was assessed based on the three point system; eggs were incubated individually. Twentysix random samples were chosen (6 from each patient). Follicular fluid (FF) was kept on ice and processed immediately as described previously (4). One ml of FF was mixed with an equal volume of 20% trichloroacetilacid (TCA). The mixture was centrifuged for 10 minutes (2000 g at 4°C) and the supernatant was extracted by chloroform. The method was essentially the same as that given by Lavy et al (5).

A 10 (il aliquots of extracted samples were analyzed by reversed phase chromatography (chromosphere C-18) HPLC using a Perkin Elmer Series 3B liquid chromatograph for delivery of solvent. Detection was performed spectrophotometrically at 260 nm using a flow-through system. Data was analyzed on-line by means of a Sigma 10B Chromatography Data Station (Perkin Elmer). A flow rate of 1.4 ml/min was used at ambient temperature and a linear separation gradient was started after 4.5 minutes. Starting conditions were 100% low strength eluant (0.02 M KH2P04, PH 5.6) and increasing to 50% strength eluant (65% methanol). Standards consisting of adenosine, adenine, xanthine, hypoxanthine, cAMP, inosine/guanine, and uric acid were used to calibrate the column daily and to establish retention times for Individual peaks. Aliquots of 10 ul of each unknown or standards were applied to the column. Peak areas were monitored on line.

The data were analyzed by using Student's t-test, and analysis of variance (ANOVA) when appropriate. Spearman rank correlation and multiple regression were used to determine the correlations between egg maturity, fertilization and purine levels. The level of significance was p<0.05.

RESULTS

The meantSD number of follicles aspirated was 16.5±2.5 (14-20) per patient. Total of 45 oocytes were obtained from the four patients studied. The mean estradiol level on the day of hCG was 920±64.5 pg/ml. All the patients received their hCG on the days 9-10 of their induction cycle. The fertilization rate was similar between the patients (Table 1).

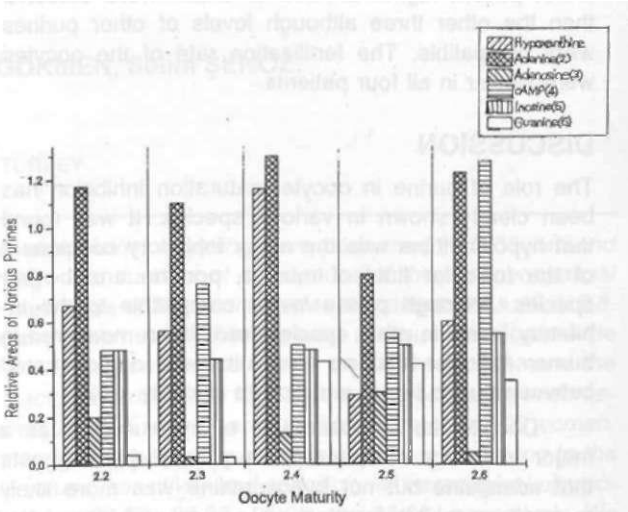

Figure 1. Relative areas of various purines in each follicle correlated with oocyte maturity (p>0.05, by ANOVA)

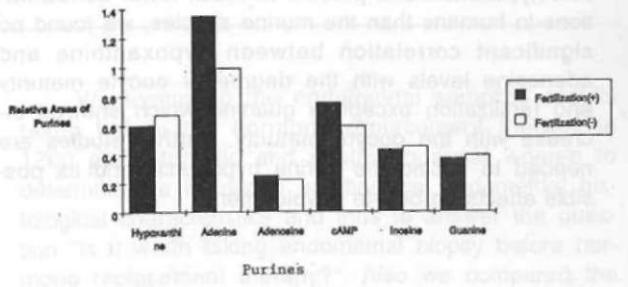

Figure 2. Relative areas of various purines in each follicle in relation to fertilization (p>0.05, t-test)

The amounts of each purine analyzed by HPLC are expressed as relative peak area levels are .shown in Figure 1,2 in terms of oocyte maturity and fertilization. Six different peaks were identified in each sample. Hypoxanthine and cAMP was present in low concentrations while adenosine were detected in only a limited number of the follicles from each patient. There were no significant differences in the purine levels in the oocytes of different maturation stages except in the case of guanine levels which was noted to

show a tendency of increase in the more mature oocytes (2.4-2.6). There were no significant difference in the levels of purines in relation to fertilized or unfertilized oocytes. There was no apparent correlation with adenosine content and degree of oocyte maturation, fertilization, or embryo quality ($p>0.05$).

In three of the four patients there were no differences between the relative levels of purines. In the fourth patient higher amount of cAMP were detected than the other three although levels of other purines were compatible. The fertilization rate of the oocytes were similar in all four patients.

DISCUSSION

The role of purine in oocyte maturation inhibition has been clearly shown in various species. It was found that hypoxtanthine was the major inhibitory component of the follicular fluid of murine, porcine and bovine species. Although purine levels compatible to the inhibitory levels in other species have been measured in human follicular fluid, no correlation was demonstrated between purine levels and oocyte maturity (2-4).

Despite the proposed role of hypoxanthine as a major inhibitory component, Lavy et al (5) suggests that adenosine but not hypoxanthine was more likely to exert an inhibitory effect on oocyte maturation. We have found measurable amounts of various purine in the human follicular fluid which is consistent with the literature supporting the purine hypothesis.

While we agree with previously published reports that hypoxanthine is present in much lower concentrations in humans than the murine species, we found no significant correlation between hypoxanthine and adenosine levels with the degree of oocyte maturity and fertilization except in guanine which showed increase with the oocyte maturity. Further studies are needed to outline the purine hypothesis and its possible effects on oocyte development.

İnsan follikül sıvısında adenozin metabolitlerinin araştırılması

Pürinlehn in vivo ve in vitro olarak mayoz bölünmeyi durdurduğu düşünülmektedir. Bu prospektif çalışmada suni olarak IVF-ET siklusları uyarılan hastaların follikül sıvılarında elde edilen pürinlerin oosit olgunlaşması ve döllenme için bir belirleyici olup olamayacaklarını araştırdık. Dört hastadan alınan çok sayıda follikül sıvısında hipoksantin, adenin, adenozin, cAMP, inozin ve guanin miktarları HPLC metoduyla tayin edildi. Purinler ile oosit olgunlaşması ve döllenme hızı arasında yalnızca guaninin olgun oositlerde daha fazla miktarda bulunmasının dışında muhtemel bir ilişki izlenemedi. [TurkJMedP.es 1995, 13(3): 94-96]

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