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Letters to the Editor

ADA (22G>A) polymorphism: a possible genetic marker for predictive medicine of human reproduction?

To the Editor:

The article by Wen and collaborators [1] published in the *Metabolism* entitled "High follicular fluid adenosine levels may be pivotal in the metabolism and recycling of adenosine nucleotides in the human follicle" is a very interesting article highlighting the crucial role played by adenosine levels in oocyte survival. This study indicates that functional ADA is absent from the follicular environment of the maturing oocyte. Interestingly, they also suggest that the ova failing to fertilize express ADA (albeit at low levels) and thus alter the composition of the follicular fluid environment by activation of pathways that result in adenine conversion to uric acid; this phenomenon would appear to be detrimental to oocyte viability, leading to the reduction of the energy availability charge of the unfertilized ova at ovulation.

Given this evidence, the authors speculate on a much broader role for adenosine in follicular fluid oocyte physiology and suggest that further study of this phenomenon (and associated pathways) may yield markers useful in the selection of oocyte for assisted reproduction beyond the current sole criterion of follicular size.

In the reproductive system, adenosine metabolism regulation seems to have a fundamental importance for the maintenance of a normal pregnancy. In particular, experimental evidence suggests that ADA placental activity is of paramount importance for normal pregnancy [2]. Furthermore, it is well known that many knockout models for *ADA* gene suffer from early lethality; and recently, a mouse model for *ADA*-SCID has been obtained through genetic manipulation to maintain ADA expression at the placental level only [3]. Recently, a reduced activity of plasma adenosine deaminase in normal pregnancy has been reported [4].

However, the importance of adenosine levels in human fertility has been highlighted since the early 1980s by numerous genetic studies. In humans, *ADA* is a polymorphic enzyme due to a G to A transition at nucleotide 22 of exon 1 that gives rise to an Asp (allele 1*) to Asn (allele 2*) amino acid substitution at position 8 of the mature protein. The enzymatic activity of *ADA**2 is approximately 35% lower than that of the *ADA**1 allele [5]; and consequently, carriers of *ADA**2 allele show a higher level of both circulating and intracellular adenosine. To date, several studies have reported significantly lower frequencies of *ADA**2 carriers in couples with sterility [6], in women with recurrent spontaneous abortion [7], in those with a high variability in length of gestation [6], and in low–birth weight infants [6]. On the other hand, in diabetic pregnancy, the proportion of *ADA* *2/*1 nonmacrosomic newborns delivered by *ADA* *1/*1 mothers is significantly higher than expected [8]. Moreover, in relation to ABO fetomaternal compatibility status, incompatible infants show a higher proportion of *ADA* *2/*1 as compared with compatible newborns [6]. Of note, a significant correlation between birth weight and placental weight has been found in subjects carrying *ADA**2 allele [9].

Therefore, in light of these evidences, we strongly suggest that adenosine levels are critical not only for oocyte maturation but also for intra- and extrauterine selection. Overall, the data reported by Wen et al [1] seem to further support the role of *ADA* genetic polymorphism on human reproduction and call for future research in this area. We may speculate that *ADA* polymorphism could be considered, in the future, an important parameter in clinical practice and genetic counseling either for in vitro fertilization programs or for the control of pregnancy outcome, further improving maternal and newborn health care.

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Reply: Purine metabolite levels in preovulatory human follicles may hold the key to ovarian hyperstimulation syndrome

To the Editor:

We are in agreement with Valerio Napolioni that adenosine's importance in reproductive biology may be wider and more variable than the association with energy charge that we describe. Indeed, we feel that the potent pharmacology of this purine may be unwelcome under emerging circumstances. Tremendous progress has been made in assisted reproductive technology since the birth of the first baby in 1978 by in vitro fertilization (IVF). The development of intracytoplasmic sperm injection in the early 1990s further increased the pregnancy and live birth rates [1]; nonetheless, a serious iatrogenic illness arose from this technology in the form of ovarian hyperstimulation syndrome (OHSS). This is experienced by approximately 5% to 10% of women undergoing IVF; and the clinical symptoms of OHSS are graded mild, moderate, and severe. Mild symptoms include abdominal bloating and feeling of fullness, nausea, diarrhea, and slight weight gain. The progression to moderate symptoms is defined by excessive weight gain (weight gain of >2 lb/d), increased abdominal girth, vomiting, diarrhea, concentrated urine, and excessive thirst. Severe symptoms are marked abdominal distension due to ascites, pulmonary edema, and chest pain [2].

The molecular cause of OHSS has been put down to a soluble factor found to be produced by multiple follicles that arise as a result of deliberate ovarian stimulation. Research into follicular fluid has been undertaken in many different ways: immunoassays for specific molecules or hormones [3-5], proteomic studies by 2-dimensional electrophoresis and mass spectrometry analysis [6], and granulosa cell messenger RNA quantification for inhibin-activin-follistatin system by polymerase chain reaction [7]. The primary focus in the search for the molecular agent responsible for OHSS has centered on vascular endothelial growth factor (VEGF), perhaps because of our current preoccupation with genes and proteins rather than smaller potent bioactive molecules. There is a presumption that VEGF levels are supraphysiologic in follicular fluid and will cause local blood vessels to become leaky [2]. Unfortunately, VEGF levels are not particularly elevated in follicular fluid compared with other sources [8]. However, smaller vasodilatory purine metabolites are present in follicular fluid in abundance [9]. In the 1980s, Downs et al [10] studied their roles as meiotic inhibitors (in mice predominantly). Later, Lavy et al [11], studying purine metabolite levels in human follicles from both natural and stimulated cycles, claimed that adenosine was the inhibitor of human oocyte maturation. In our recent study, we found that hypoxanthine levels were extremely variable, but adenosine was a consistent component, and levels were supraphysiologic—the smaller follicles contained a much greater concentration of adenosine than the larger ones [9].

Adenosine's other biological actions make it a significant contender as the molecular cause of OHSS: adenosine is a powerful vasodilator; and when administered by intravenous infusion, it can produce substantial hypotension. Acting via adenosine A2 receptors, it induces smooth muscle relaxation, especially in the coronary circulation; but because of its extremely rapid metabolism, it is very short acting. It is common practice to infuse adenosine into coronary arteries when imaging occlusions, but it is not used clinically as a vasodilator. However, in such patients, most of adenosine's side effects are related to its vasodilatory properties. Furthermore, peripheral microvascular endothelia local production of vascular permeability factor/VEGF A is up-regulated 2- to 3-fold by adenosine [12].

A wave of symptoms due to vasodilation and increased vessel permeability spreading from the ovaries to the abdomen and on to the lungs would be consistent with unopposed peritoneal infusion of adenosine (leaking from multiple follicles). This is prevented by adenosine deaminase, or ADA (also referred to as *adenosine aminohydrolase*, EC 3.5.4.4), a ubiquitous enzyme that appears to be particularly important in the development of thymocytes. ADA converts adenosine into inosine through the hydrolysis of the purine amino group, with an estimated half-life of 1 second (Fig. 1). ADA is present in all tissues, but activity is particularly high in thymocytes of the thymic cortex. There are 2 enzymes that carry out ADA activity, called *ADA1* and *ADA2*. ADA1 a 40-kd monomeric protein with a 200-kd noncatalytic combining protein, and it is responsible for about 90% of adenosine deamination. ADA2 is somewhat larger at 110 kd and appears to play a general adenosine deamination role in serum. Total absence of ADA activity results in a form of severe combined immunodeficiency.