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GAMETE BIOLOGY

Sperm nucleus decondensation, hyaluronic acid (HA) binding and oocyte activation capacity: different markers of sperm immaturity? Case reports

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Introduction

During the early time of IVF, sperm competence was defined as the ability to fertilize an oocyte. However, with the advent of ICSI, despite the capacity to reach 2–4cell stage, it is impossible for numerous patients, to establish a pregnancy. Instead the consensus now is that male fertility has to be defined as the capacity to produce sperm cells able to establish a full term pregnancy [1–3]

Capsule Based on several observations in infertile couples, we have found that immaturity based on HA binding and nucleus decondensation have not exactly the same feature. However both anomalies can be cured by manipulation of the one carbon metabolism, improving homocysteine recycling i.e. vitamins of the group B and Zinc. Our observations confirm that in human sperm, fertility potential is highly dependent of methylation.

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Basic parameters such as concentration, motility and morphology are of limited value in determining sperm capacity to allow full embryonic development to term. Determination of DNA changes like fragmentation and condensation are independent parameters [2] and obviously of major importance. DNA fragmentation, related or not to reactive oxygen species (ROS) insult is only one piece of the problem. Indeed, sperm chromatin tertiary structure seems to be critical for correct epigenetic regulation and maintenance, and further on for male fertility [4, 5]. During very early embryogenesis, an adequate chromatin structure is also necessary for the very first cleavages [6–9]. Methylation is one of the most important regulators of the tertiary structure and imprinting; it affects sperm DNA and histones packaging it; in this way a correct recycling of homocysteine is mandatory during spermatogenesis and embryogenesis. The sperm is not just a carrier of paternal genome: it has a relevant epigenetic contribution. A defective wrapping of DNA is often linked to immaturity. Hyaluronic acid (HA) binding ability has been proposed as a tool to select the more mature spermatozoa having reached their final nuclear and cytoplasmic maturation [10, 11], even if controversies exist [12], and it is used as well in veterinary medicine [13]. In this case report we have tested the relation between HA binding and methylation effector supplementation, by comparison to what we have observed with such a supplementation for patients with poor sperm condensation.

Case report

We report the case of the couple A, who had primary infertility since 2004. Mrs A was born in 1970 and her

husband was born in 1966 . They consulted in 2004 for the first time. The female patient had regular menses, FSH at 5.4 UI/mL, estradiol at 53 pG/mL, AMH at 2nG/mL and a normal hysterosalpingography. Her husband had a rather normal semen: 1.5 mL, 100 millions/mL, 40% motility. Both karyotypes are normal.

We started a first IVF cycle in June 2008, using a long agonist protocol. 4 oocytes were retrieved and no was fertilized following a classical IVF; Following this failure, a ICSI attempt using HA (Spermslow, Medicult Danmark) was performed in October 2008. No binding to Spermslow was observed and the sperm was tested for fragmentation (DFI: 36%, threshold negative value >30%) using the TUNEL assay and the nucleus condensation (SDI: 10%, threshold negative value >25%) using Aniline blue (for details, see Belloc et al. 2009). The male patient took orally, one pill of Condensyl®,(Nurilia France). Each pill of Condensyl contains quercetin (25microgram), a polyphenol sustaining the germ line, all the vitamins of group B (B2:1.4 mG, B3: 16 mG, B6: 2 mG,B9: 200 μG, B12:1 μG) and Zn:15 mG, involved in recycling of homocysteine. In May 2009, 6 months after the beginning of treatment, the HA binding capacity was recovered (>50%), DFI was not decreased (36%) and SDI was found at 15%. In September 2009, after continuing the treatment, we found a DFI of 18%, and a SDI at 12%. Binding to hyaluronic acid was found normal at this time (>50%).

Based on these positive results, the couple started then a COH (long acting analog) protocol followed by an IMSI (using Spermslow for sperm selection and injection). 3 oocytes were retrieved, but no pronuclei formation was observed. Another attempt was proposed to the couple; it was performed in April 2010: 6 oocytes were retrieved followed by another IMSI. And again no pronuclei formation was observed. The unfertilized oocytes were cytogenetically analyzed: A premature chromosome condensation, associated with the absence of expulsion of the second polar body was observed. The diagnosis was an absence of oocyte activation. The couple was advised that an ICSI attempt should be performed with a concomitant mechanical activation of the oocyte. The couple after a reflexion and information period, came back to us for another IMSI cycle in July 2010: the simultaneous activation (by sham injection) allowed the obtention, for the first time, of one embryo out of the 4 oocytes. Based on this observation the couple wanted to make another (last) attempt. Sham injection is the only activation process authorized by the French authority (Agence de Biomedecine); the use of Ca ionophore is forbidden.

After stimulation, 7 MII oocytes were collected, denuded and activated by a sham injection, followed 30 min later by a classical IMSI injection with HA. One oocyte showed the following day, the presence of 2 pronuclei. The corresponding embryo was transferred on D2. A female baby of 3 kG was delivered without any problem (Apgar 9), in September 2011.

Discussion

Sperm decondensation and absence of HA binding are two different aspects of "immaturity". In our activity when isolated decondensation is established (no DNA fragmentation), the patients are systematically treated with the association of Zinc+vitamins B and quercetin (Condensyl®). The increase in condensation occurs between 3 and 6 months and the mean increase in condensation is 30% after 4 months (range 3–80%, in press).The treatment is followed by pregnancies after regular ICSI, artificial insemination or even spontaneous pregnancy. This is not the case for the couple described here. Activation capacity is strictly related to phospholipase zeta [14, 15].The lack of HA binding does not seem to cover only sperm immaturity: the situation seems more complex here. An additional interesting observation is that our treatment of Zn and vitamins of the group B are able to rescue the HA binding as it does for the DNA packaging. In any case, our observations confirm that in human sperm, fertility potential is highly dependent of méthylation [16, 17] the major non-genetic contributions of the sperm nucleus to embryonic development. This observation confirms that homocysteine recycling i.e. one-carbon metabolism is the epicenter of human infertility [18]

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