Hyaluronan in follicular fluid and embryo implantation following in vitro fertilization and embryo transfer

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Abstract

Purpose To determine whether the concentration of hyaluronan (HA) in follicular fluid predicts implantation success following embryo transfer.

Methods Follicular fluids from 170 IVF patients were tested by ELISA for HA concentration.

Results The mean (standard error) HA concentration in follicular fluids was 158.0 (21.9) ng/ml from women whose embryos did not implant, 220.0 (21.3) ng/ml from women in which one embryo implanted and 239.3 (40.1) ng/ml from women with 2–3 implantations (implantation vs. no implantation $p=.019$). The HA level was unrelated to maternal age, number of oocytes harvested or fertilized or number of embryos transferred. Follicular fluids from women with an endocrine problem had a lower mean HA level (142.0 ng/ml) as compared to women undergoing IVF due to male factor infertility (257.3 ng/ml) ($P=.05$).

Conclusions HA in follicular fluid is decreased in women with unsuccessful implantation or with an endocrine disorder. A woman's level of HA production may influence the potential for implantation of her embryos.

Keywords Hyaluronan · Implantation · Infertility · In vitro fertilization

Background

Implantation of the embryo is an important rate limiting step in in vitro fertilization. The factors influencing whether or not an in vitro fertilized embryo will successfully and effectively attach to the uterine wall, as well as the variables favoring multiple implantations, remain inadequately delineated. Better elucidation of the mechanisms involved would enable clinicians to more efficiently modulate conditions so as to maximize the likelihood of a successful IVF outcome in individual women while minimizing the chances for a multifetal pregnancy.

Hyaluronan (HA) is a large glycosaminoglycan composed of repeating disaccharides of D-glucuronic acid and N-acetyl glucosamine and is a component of the extracellular matrix of the reproductive tract. It is synthesized by cumulus cells in response to a luteinizing hormone surge or to chorionic gonadotropin administration [1]. HA production is necessary for detachment of the cumulus-oocyte complex from the follicle wall. Some of the HA becomes a component of the follicular fluid. It has been suggested that the follicular fluid HA level may indicate follicular maturity, but the conclusions have been conflicting [1, 2].

In laboratory mice, HA synthesis in the uterus is markedly increased at the time of implantation [3], suggesting the involvement of HA in this process. Furthermore, HA has been shown to facilitate development of the in vitro fertilized mouse fetus after embryo transfer [4] as well as promoting angiogenesis [5]. The primary cell surface receptor for HA, CD44, is expressed on the surface of blastocysts [6] and in secretory phase endometrium [7].

Capsule Women with successful implantation following embryo transfer have higher levels of hyaluronan in their follicular fluid than women whose embryos do not implant.

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Supplementation of embryo transfer medium with HA has been shown to increase the rates of implantation, clinical pregnancy and multiple pregnancies [8–10].

The possible influence of HA concentration on oocyte maturation and/or implantation potential prompted us to evaluate for an association between the level of HA in follicular fluid and various clinical parameters in women undergoing a cycle of in vitro fertilization-embryo transfer.

Methods

We performed a blinded retrospective study of HA concentrations in follicular fluids from 170 women who underwent a first, second or third cycle of in vitro fertilization-embryo transfer in the Division of Reproductive Endocrinology and Infertility in Dortmund, Germany. All women underwent the identical stimulation protocol. All subjects signed written informed consents to participate in the study. Gonadotropin-releasing hormone agonist (Synarela, Pfizer, Karlsruhe, Germany) was given continuously beginning in the mid-luteal phase of the prior cycle. After down-regulation was complete, gonadotropin was administered beginning on the second day of the stimulation cycle until administration of 10,000 IU hCG (Predalon, Organon, Munich, Germany). For most patients a stimulation protocol was applied that involved the combined application of recombinant FSH (Puregon, Organon, Munich, Germany, Gonal F, Merck-Serono, Darmstadt, Germany) and highly purified hMG (Menogon HP, Ferring, Kiel, Germany). The dosage of gonadotropins was titrated by estradiol concentration and follicle size measured by transvaginal ultrasound. Ovulation was induced when ultrasound verified that at least three follicles were 17 mm in diameter. Ovulation was only induced when follicle size and estradiol level simultaneously indicated the presence of a sufficient number of mature oocytes. Maturity was assumed when at least 150 pg/ml estradiol per measured follicle was present. There was no difference in the level of estradiol among all investigated groups. Transvaginal oocyte retrieval and follicular fluid collection was initiated 34–36 h following ovulation induction, as reported previously [11]. After oocyte retrieval, the cumulus-oocyte complexes were collected in Gamete medium (Vitrolife, Göteborg, Sweden) and incubated at 37°C with 5% CO₂. For the IVF procedure, the complexes were admixed with 150,000 motile sperm in 70 µl IVF medium (Vitrolife) and covered with mineral oil. For ICSI, cumulus cells were removed by hyaluronidase treatment (Vitrolife). Only mature metaphase II oocytes were used for ICSI. After the injection of a single spermatozoa, the oocytes were placed in 70 µl IVF medium and covered with mineral oil. Survival and fertilization were controlled after a 16–18 h

incubation at 37°C in 5% CO₂ by ascertainment of the presence of two pronuclei. Fertilized oocytes were transferred to fresh IVF droplets and incubated an additional 48–60 h. On day 2 or 3, the transfer of embryos was performed in IVF medium. The IVF medium is designed for fertilization, embryo culture as well as embryo transfer. Therefore, it was used according to the manufacturer's instructions as both an IVF medium and a cleavage medium. There was no difference in the number of embryos transferred or pregnancy rates in our subjects between day 2 and day 3 transfers. This agrees with others' reports [12]. The average number of embryos transferred was two for women < 35 years of age and 3 for those > 35. The primary cause of infertility was obtained from each woman's medical records. Tubal occlusion was diagnosed in all cases by laparoscopic examination. The clinical pregnancy rate at the time of this study was 32% per embryo transfer.

Follicular fluids were aliquoted and frozen at –20°C following collection. A convenience sample from women with either 0, 1, 2 or 3 embryo implantations was forwarded to Cornell on dry ice and assayed as a single batch. Samples were thawed, diluted 1:4 in reagent diluent and tested in duplicate for HA concentration by a commercial ELISA (R & D Systems, Minneapolis, MN). Values were converted to ng/ml by reference to a standard curve that was generated in parallel to the test samples. Laboratory personnel were blinded to all clinical data and IVF outcome.

The relationships between HA concentration and numbers of oocytes retrieved and fertilized, embryos transferred and implanted, and cause of infertility, as well as the association between maternal age and number of implantations, were analyzed by the Mann-Whitney test, since the data were not normally distributed. The possible association between maternal age and HA concentration was analyzed by the Spearman rank correlation test. Statistical analyses were performed using GraphPad Software (San Diego, CA.)

Results

One to six oocytes were retrieved from 17.7% of the patients, 7–12 were retrieved from 28.7%, 13–20 from 32.0% and >20 were harvested from 21.5% of the patients. A single oocyte was fertilized in 5.8% of the women, 2–5 were fertilized in 34.0%, and >6 oocytes were fertilized in 60.2% of the women. There were no associations between the number of oocytes retrieved or fertilized and the follicular fluid HA concentration. Also, there were no differences in the number of implanted embryos when either 2 or 3 embryos were transferred.

Follicular fluids from 43 women (mean age 33±4 years) whose embryos failed to implant, 65 women (mean age 31±4 years) in which a single implantation occurred and 62

Fig. 1 Association between concentration of hyaluronan in follicular fluid and subsequent embryo implantation. Follicular fluids from 170 IVF patients whose embryos failed to implant after transfer or where one or two–three embryos implanted were tested for levels of hyaluronan by ELISA

women (mean age 31 ± 4 years) with 2–3 implantations were evaluated for HA concentration. The difference in ages between groups was not statistically significant.

The results of measurement of HA concentration are shown in Fig. 1. The mean follicular fluid HA concentration (standard error) increased from 158.0 (21.9) ng/ml in women whose transferred embryos did not implant to 220.0 (21.3) ng/ml in women with a single implanted embryo to 239.3 (40.1) ng/ml in women with multiple implantations. Only the differences in HA concentration between implantation failure and successful implantation were significant ($p=0.019$). A spontaneous abortion occurred in 6.5% of women with successful implantation. The mean (standard error) follicular fluid HA level was 189.8 (68.7) ng/ml in women who spontaneously aborted vs. 212.3 (19.8) ng/ml in those whose embryos did not abort. This difference was not significant.

The frequencies of the different causes of infertility in our patient population were 33.6% tubal occlusion, 23.6% endocrine dysfunction, 20.7% male factor, 15.0% endometri-

osis and 7.1% idiopathic. The follicular fluid HA results from each group are shown in Fig. 2. The women with all normal parameters (male factor infertility) had the highest mean (standard error) follicular fluid HA concentration, 257.3 (44.8) ng/ml. Only the women with endocrine dysfunction had a reduced mean (standard error) follicular fluid HA level, 142.0 (21.4) ng/ml, compared to the male factor group ($p=0.05$).

The mean (standard error) follicular fluid HA level was 255.9 (43.3) ng/ml for the 52 patients < 30 years old, 197.9 (21.6) ng/ml for the 85 women 31–35 and 142.1 (16.47) ng/ml for the 33 women 36–40 years old. These differences were not statistically significant. The follicular fluid HA level was also unrelated to body mass index or to the presence or absence of polycystic ovaries (data not shown).

Discussion

The mean concentration of HA in follicular fluid from women undergoing in vitro fertilization and embryo transfer was increased in those cases with a subsequent successful embryo implantation. This observation can be interpreted as suggesting that individuals who synthesize higher concentrations of HA as a consequence of genetic or environmental fluctuations are at a selective advantage for successful implantation. The observation that the follicular fluid HA level was unrelated to the number of oocytes harvested or fertilized or to the number of embryos that were successfully grown in vitro and subsequently transferred back to the patients suggests a lack of variation in oocyte quality or maturity. Consistent with the observation of others that supplementation of the transfer medium with HA increases the implantation rate [8–10], the finding in the present report reinforces the probability that elevated production of HA enhances the probability of successful human embryo implantation.

The mechanism whereby HA facilitates implantation remains a matter of speculation. Prior studies suggest a

Fig. 2 Association between concentration of hyaluronan in follicular fluid and cause of infertility. Follicular fluids from 170 IVF patients with various causes of infertility were tested for levels of hyaluronan by ELISA

potential role in cell-matrix interactions during blastocyst-endometrium binding [8, 13] and in facilitating development of new blood vessels [5]. Alternatively, the physical properties of HA may be responsible for its utility during implantation. Its high viscosity may inhibit expulsion of the embryo from the uterine cavity after transfer [9] as well as facilitate interaction of the embryo with macromolecules present in the lumen [10]. Embryo implantation has also been shown to be an inflammatory process requiring immune system activation and cytokine production [14]. The involvement of HA and HA breakdown products in the regulation of pro-inflammatory immunity has been demonstrated [15, 16].

To suggest from our observation of elevated HA in follicular fluid that HA is involved in the implantation process requires the assumption that the follicular fluid HA concentration is indicative of the individual's capacity for synthesis of high, intermediate or low HA levels. Further studies are required to examine whether genetic factors such as polymorphisms in the genes coding for HA synthases and/or HA receptors do indeed influence the implantation rate in women undergoing in vitro fertilization. In addition, our observation that women who seek assisted reproductive technology due to an endocrine disorder have the lowest mean follicular fluid HA concentration suggests hormonal involvement in HA production. It is interesting to speculate that one reason women with endocrine abnormalities are sub-fertile is a relative decrease in their capacity for HA production. Additional investigations of this proposed mechanism may be fruitful.

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