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Chapter 6

THE ROLE AND USE OF HYALURONAN IN REPRODUCTIVE MEDICINE

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ABSTRACT

This chapter presents the usefulness of hyaluronan in reproductive medicine.

Hyaluronan is a major constituent of the extracellular matrix of the cumulus cells in the cumulus-oocyte complex and may play a critical role in the selection of functionally competent spermatozoa during *in vivo* or *in vitro* fertilization. Hyaluronan can serve as the selective marker during intracytosplasmic sperm injection (ICSI) to select and inject the most optimal spermatozoon. The technique of ICSI requires the immobilization of the spermatozoa. Polyvinylpyrrolidone (PVP) routinely used during ICSI, facilitates handling of spermatozoa. PVP is an artificial polymer, which has been regarded as chemically inert, although adverse effects as a result of its use have been reported. Viscous solution of hyaluronan can be used as an alternative to toxic PVP. Moreover, hyaluronan is also a major glucosaminoglycan in the uterine fluid. It has been shown to increase cell-cell adhesion and may improve embryo apposition and

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attachment. Some studies suggest that the use of hyaluronan containing embryo transfer medium can improve the implantation.

INTRODUCTION

The development of the ICSI technique introduced a new approach to treating male infertility [1]. Almost 20 years later more and more studies point to the unknown side effects of the mentioned method. In the ICSI technique the spermatozoa are artificially selected, not allowing the natural selection to take place among them.

Wrong selection of a spermatozoon could cause the injection of the spermatozoon with fragmented DNA, which could later result in the decreased level of the fertilization, affect the quality of the embryo and the embryo's implantation ability [2-9] or increase the possibility of spontaneous abortion [9-14].

Studies also emphasize the higher share of children conceived by the ICSI method with congenital defects compared to children conceived by the classic IVF (in vitro fertilization) method or naturally conceived children [15-17]. The right selection of the spermatozoon is crucial for the successful outcome following the ICSI technique, therefore reproductive medicine is searching for methods which would enable a more reliable selection of spermatozoa for intracytosplasmic injection.

Hyaluronan, which is the integral part of extracellular matrix of cumulus cells surrounding the oocyte, represents such an option. Namely, only spermatozoa without DNA defragmentation, cytoplasmic retention and proper morphology has the hyaluron-binding ability [18-20]. This ability is used in reproductive medicine to select the spermatozoon for intracytoplasmic injection, for evaluating the fertilization ability of spermatozoa or explaining male infertility.

In addition to the artificial selection of the spermatozoon in the ICSI method, the use of polyvinylpyrrolidone (PVP) for the necessary immobilization of spermatozoa during the procedure also has a negative effect on the procedure's outcome. Hyaluronan as a viscous solution represents a successful substitute for toxic PVP [21, 22].

Hyaluronan is also present in cervical mucus. Using hyaluronan in the embryo transfer medium can significantly affect the embryo's successful implantation [23-26]. In the following section we present the mentioned options of using hyaluronan in reproductive medicine.

THE DEVELOPMENT OF HYALURONIC ACID IN THE EXTRACELLULAR MATRIX OF THE CUMULUS CELLS AND ITS ROLE

The human oocyte is surrounded by several layers of cumulus cells (Figure 1) which provide the necessary nourishment and hormones during its development and growth. On the luteinizing hormone, which is a signal for oocyte maturation and ovulation, numerous changes are triggered. The cumulus cells begin to build the extracellular matrix, which mostly consists of hyaluronic acid [27].

Hyaluronic acid or hyaluronan is a glycosaminoglycan, a linear polymer made of repeating disaccharide, D-glucuronic acid units (1-β-3) and N-acetyl-D-glucosamine (1-β-4). Hyaluronan is synthesised by means of integral membrane proteins called hyaluronan synthases found in three forms: HAS1, HAS2 and HAS3. Enzymes build a chain by attaching the glucuronic acid and N-acetyl glucosamine intermittently on the developing polysaccharide.

Synthesised hyaluronic acid transfers, with the help of transfer protein, through the cell membrane into the extracellular space and is thus the integral part of the extracellular matrix of the cumulus cells surrounding the oocyte [28, 29]. The molecules of the hyaluronan are in the extracellular matrix of the cumulus cells cross-connected with proteins of the extracellular matrix and proteoglycans. Such extracellular matrix of the cumulus cells develops not sooner than and only before the ovulation. Hyaluronan incorporates into the gap junctions of cumulus cells.

By doing so, it loosens the junctions between cumulus cells as well as their attachment to the oocyte [30] and enables the spermatozoon's passage through the cumulus cells to the oocyte [31].

A high concentration of hyaluronan in the follicular fluid is linked with a successfully fertilized oocyte [31].

SPERMATOGENESIS AND ACQUIRING THE HYALURONIC ACID BINDING ABILITY

The course of spermatogenesis has three stages: proliferation phase, meiotic phase, and differentiation phase (spermiogenesis). It takes place in seminiferous tubules of the testes and right at the basal lamina lays a layer of spermatogonias (with diploid number of chromosomes).

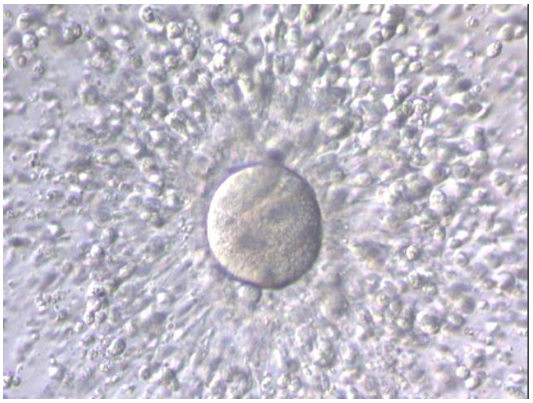

Figure 1. Oocyte surrounded by several layers of cumulus cells (oocyte cumulus complex).

Spermatogonias divide mitotically (proliferation phase). Some of the cells which are the result of spermatogonial cell division develop into primary spermatocytes. The primary spermatocytes enter into meiotic division, resulting in haploid round spermatids (meiosis). During spermiogenesis the spermatozoa develops from the round spermatid. During this process histones are eliminated and replaced by protamines.

Moreover, a part of the nuclear membranes, half of mitochondria and a larger part of cytoplasm are discharged, an acrosome develops, and receptors are built into the membrane for binding to zona pellucida and binding to hyaluronan.

As the receptors for binding to zona pellucida and binding to hyaluronan have the same origin and are built into the membrane simultaneously, the two characteristics are closely connected [32].

THE USE OF HYALURONAN AS A SELECTIVE MARKER FOR SELECTING THE OPTIMAL SPERMATOZOON FOR INTRACYTOSPLASMIC INJECTION

The Importance of Selecting the Most Optimal Spermatozoon

In procedures for treating infertility with IVF we use two methods for fertilizing the oocytes; the intracytoplasmic sperm injection (ICSI) (Figure 2) and the classic *in vitro* fertilization (IVF) method.

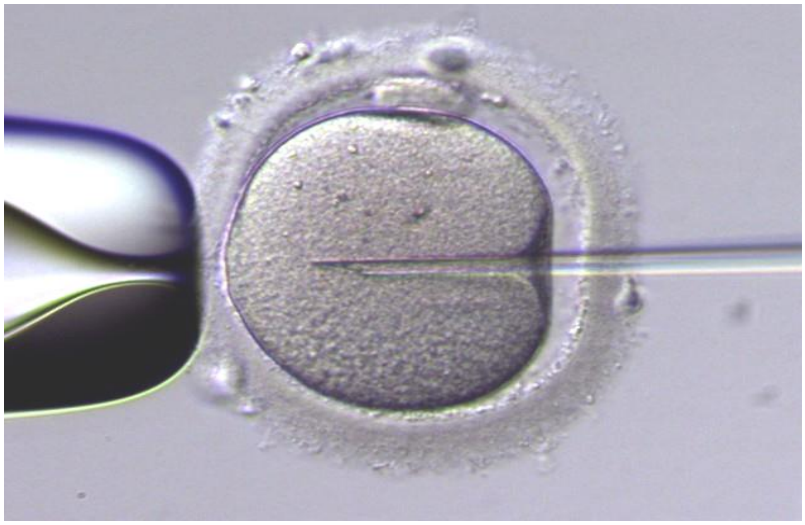

Figure 2. Intracytosplasmic sperm injection (ICSI).

In the latter, we only bring the spermatozoa closer to the oocyte and they must come into the oocyte by himself. In the ICSI technique we physiccally deposit the spermatozoon (with the injection needle) into the cytoplasm of the oocyte.

Compared to IVF, the ICSI technique provides a higher share of fertilized oocytes and lower probability of total fertilization failure [33-35]. But on the other hand, in the IVF method we allow the natural selection between spermtozoa to take place, whereas in the ICSI technique, the selection is performed by an embryologist, who first provides a subjective evaluation of motility and morphology of the spermatozoon and then selects the most appropriate one. The success of the ICSI technique mostly depends on the selection of the spermatozoon injected.

The spermatozoon´s hyaluron-binding ability is one of the biomarkers based on which we select the appropriate spermatozoon. This selection is based on the fact that the spermatozoon requires this ability to recognize and bind to hyaluronan also *in vivo* for successful fertilization [36]. Only mature spermatozoon binds to hyaluronan (through receptors on its surface), with normal chromosomal status, without retention of cytoplasm, the remaining unreplaced histones (residual histones) or apoptotic markers [19, 37] and without fragmented DNA [2, 20]. The most important characteristic of spermatozoon injected into oocyte is the integrity of spermatozoon's DNA, which is not visible to the embryologist who selects the spermatozoon on the

basis of motility and morphology and is not reflected in the spermatozoon's altered morphology.

Avendano et al. [38] proved that even seemingly morphologically normal spermatozoa can have fragmented DNA. The consequences of inserting a spermatozoon with fragmented DNA are usually seen no earlier than after three days of embryo cultivation.

A high share of spermatozoa with fragmented DNA is related to recurring unsuccessful IVF procedures [2-9] and a higher share of spontaneous abortions [9-14]. Fertilizing the oocyte with a spermatozoon with fragmented DNA, if it happens at all, affects the embryo's vitality in later stages of development (prior to implantation and after it).

The mentioned effect is especially noticeable in embryos developing after the applied ICSI method where on account of the artificial selection of sperm the probability of injecting a spermatozoon with fragmented DNA is higher than in the IVF method [39]. Studies point to a higher share of children conceived by the use of the IVF method or naturally conceived children[15-17]. Zona pellucida has the ability to select among optimal and non-optimal spermatozoa *in vivo* [40, 41]. The spermatozoon without the hyaluronic-binding ability could never fertilize an oocyte *in vivo* as it does not have the ability to bind to zona pellucida which is unavoidable in the fertilization process (Figure 3). Thus the spermatozoon's ability to bind to hyaluronan defines its fertilizing ability and the selection for injecting the spermatozoon into the oocyte during the ICSI procedure becomes very similar to *in vivo* selection. Thus two laboratory techniques have been developed. The first one enables the selection of sperm for intracytoplasmic injection and the second one assesses the spermatozoa's fertilizing ability (hyaluronan-binding test).

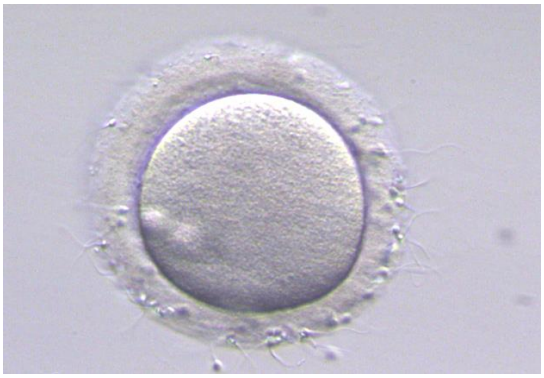

Figure 3. Spermatozoa binding to zona pellucida.

Selection of Spermatozoon for Injection into the Oocyte during the ICSI Procedure on the Basis of Spermatozoon's Hyaluronan-Binding Ability

In this method we use a Petri dish with hyaluronic acid bound to it. This method enables us during the ICSI procedure to immobilize the mature spermatozoa binding to hyaluronan [42]. The share of aneuploid spermatozoa binding to hyaluronan on the Petri dish is 4-6 fold lower in comparison to the fraction obtained after a swim-up method, widely used for sperm preparation [18]. The use of hyaluronan for selecting spermatozoa intended for the ICSI technique increases the level of oocyte fertilization [43], embryo quality [44], and pregnancy rate as well as lowers the occurrence of spontaneous abortions in comparison to patients where the selection of spermatozoa for intracytoplasmic injection was performed on the basis of the embryologist's assessment [43].

Hyaluronan Binding Test

The spermatozoa's ability to bind to hyaluronic acid is tested on special glass slides coated with hyaluronic acid (Figure 4). The test is available on the market under the name Hyluronan binding assay (HBA®).

To perform the test, 10 microlitres of a sample are required and the result is visible after 10 minutes. Bound spermatozoa are differentiated from unbound spermatozoa by their beating tails with heads that make no progressive movement. Non-binding motile spermatozoa swim about freely.

Figure 4. Glass slides for performing the hyaluronan binding test [45].

The test result is related to the spermatozoa's fertilizing ability and fertilization rate following the IVF method. The hyaluronan binding ability correlates with the percentage of morphologically optimal spermatozoa [36].

The Use of Hyaluronan Binding Test for Establishing the Spermatozoa's Fertilizing Ability and Differentiating among Samples Appropriate for the IVF or ICSI Method

The criteria for evaluating the quality of spermatozoa, is a classic semen analysis, which evaluates the concentration, motility and percentage of morphologically optimal spermatozoa. Based upon the obtained values we decide for one of the two methods for fertilizing the oocytes.

The ICSI technique is indicated only in cases with severe forms of male infertility (heavily decreased sperm concentration or motility, globozoospermia, complete absence of spermatozoa in the ejaculate, immunological cause of infertility) or in special cases when it is clear from previous IVF attempts that the percentage of fertilized oocytes after the IVF method was extremely low. The percentage of couples with one of the mentioned causes of infertility is around 30 % [47]. Despite the mentioned indications for employing the ICSI technique, this method is used in IVF procedures across Europe in almost 70% of all assisted reproduction techniques (ART) cycles [48]. The reason lies in the fact that despite normal values resulting from the classic semen analysis, the male infertility factor cannot be excluded [49-54]. In recent years a need for additional testing was created to obtain a more reliable assessment of the fertilizing ability and functional maturity of spermatozoa.

In the following section we will describe the study conducted at the Department of Reproductive Medicine and Gynaecologic Endocrinology of the University Medical Centre Maribor, Slovenia. The purpose of this study was to establish whether it is possible to estimate of spermatozoa's fertilizing ability based on the hyaluronan-binding test and thus differentiate among semen samples suitable for one of the two insemination methods.

Methods

Group of Patients

In this study we included couples who were in the first or second procedure of treating infertility with ART and where we collected at least 6 oocytes after ovarian stimulation with an ultrasound-guided ovarian puncture.

Women were younger than 37 years and the cause of infertility was not endometriosis, polycystic ovaries (PCO) or polycystic ovary syndrome (PCOS). The couples with an identified severe form of male infertility were not included in the study. A half of the oocytes were fertilized by the ICSI technique and the other half with the IVF method.

Analysis on a Sperm Sample

Semen samples were collected on the day of the ultrasound-guided ovarian puncture by masturbation after two to five days of sexual abstinence.

The semen was prepared with the density gradient technique (Puresperm, Nidacon, Švedska) and afterwards with the swim-up method. The supernatant with progressively motile spermatozoa was used for insemination of oocytes and performance of hyaluronan-binding test.

Insemination of Oocytes and Cultivation of Embryos

Half of the oocytes were inseminated with the ICSI technique and the other half with the classic IVF method. The inseminated oocytes were incubated for 18-20 hours at 37 °C, 95 % humidity, 6 % CO_2 and 5 % 0_2.

The following day the zygotes were moved to a sub-cultivation where we separated the correctly fertilized from incorrectly fertilized or unfertilized oocytes. We identified oocytes as correctly fertilized if they had after 18-20 hours visible two pronuclei (2PN) in the cytoplasm of the oocyte.

Results

133 couples successfully completed the study. The hyaluronan-binding test in 133 prepared semen samples reached values from 15% to 100% (with the average value 91.3 ± 10.9) (Figure 5).

The Effect of the Share of Spermatozoa with Hyaluronan-Binding Ability on the Fertilization of Oocytes After the IVF Method

We have established that the percentage of spermatozoa with hyaluronan-binding ability is linked with the percentage of fertilized oocytes following the IVF method (R=0.321, P=0.000) (Figure 6).

After the ICSI method we did not establish that the percentage of spermatozoa with hyaluronon-binding ability would affect the fertilization of oocytes (R=0.114, P=0.190).

The reason probably lies in the fact that the ICSI method bypasses the natural course of oocyte fertilization, more specifically, the entrance of the spermatozoon into the oocyte.

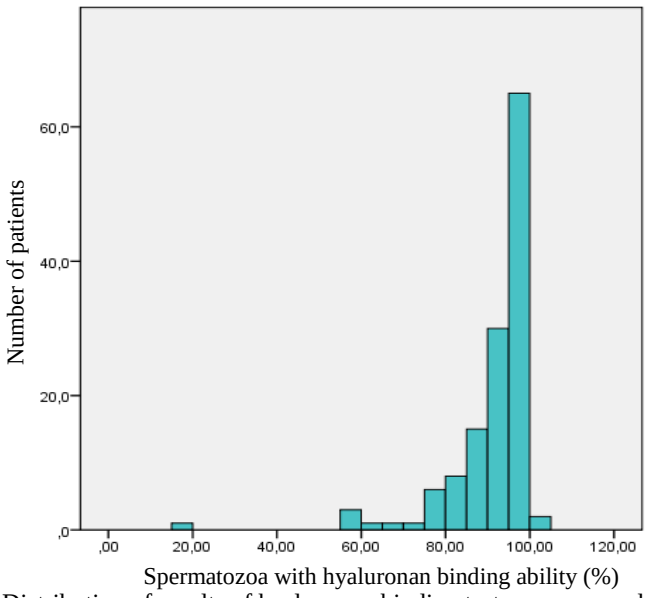

Figure 5. Distribution of results of hyaluronan-binding test on a prepared semen sample in the study male group (N = 133).

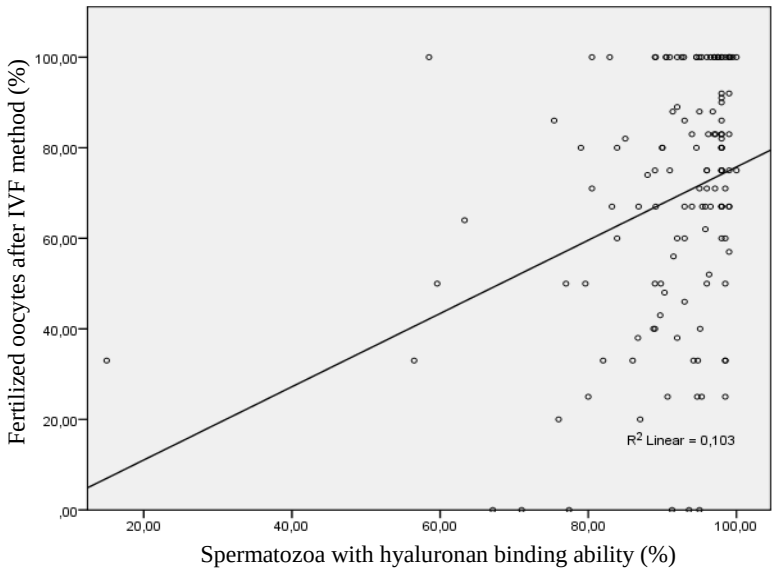

Figure 6. Percentage of fertilized oocytes following the IVF method in 133 couples regarding the percentage of spermatozoa bound to hyaluronan in a prepared semen sample.

This way the spermatozoon does not acquire the hyaluronan-binding ability as it is mechanically injected into the cytoplasm of the oocyte. Our results are in accordance with certain other studies [2, 36, 44, 55].

A Comparison of Groups with the Fertilization Rate of Oocytes Following the IVF Method of Less Than 50% and More Than 50%

Couples included in the study were divided into two groups. Group 1 was represented by couples with a fertilization rate of less than 50% following the IVF method and Group 2 was presented by couples with a fertilization rate of more than 50% following the IVF method. The groups did not differ regarding the characteristics which could significantly affect the fertilization of oocytes, i.e. mean age of women, number of oocytes collected with ultrasound-guided ovarian puncture, women's BMI or quantity of applied gonadotrophins for ovarian stimulation.

Table 1. Comparison of groups with the fertilization rate following the IVF method less (Group 1) and more than 50% (Group 2) in characteristics of the groups and characteristics of the semen

	Group 1 Fertilization rate < 50 % (N = 29)	Group 2 Fertilization rate ≥ 50 % (N = 104)	*P* value
Group characteristics			
Woman's age	31.5 ± 0.6	32.3 ± 0.3	NS
Woman's BMI	22.3 ± 0.6	22.1 ± 0.3	NS
Number of used gonadotrophin ampoules	23.5 ± 1.3	23.8 ± 0.6	NS
Number of collected oocytes	12.5 ± 1.4	13.4 ± 0.7	NS
Native semen sample			
Concentration (*10^6/ml)	44.1 ± 8.5	64.2 ± 4.7	0.045
Progressive motility (%)	30 ± 2.1	35.1 ± 1.3	NS
Morphologically normal shape (%)	5.2 ± 0.7	6.4 ± 0.4	NS
Prepared semen sample			
Hyaluronan-binding ability (%)	85.1 ± 3.1	93 ± 0.8	0.019

Values are expressed as the mean value ± *standard error mean, SEM*).
P value = measure for statistical significance, $P < 0{,}05$ statistical significance.
NS = difference not statistically significant.

There were also no differences between the groups in the quality of the native semen sample (motility and percentage of morphologically normal shapes). The only slight difference was in the sperm concentration in native samples (P = 0.045).

The groups differed in statistical significance regarding the percentage of spermatozoa with hyaluronan binding ability (P = 0.019) defined in a prepared semen samples used for later insemination of oocytes (Table 1).

By using the a receiver operating characteristic (ROC) curve we set the limit value for the percentage of spermatozoa with hyaluronan-binding ability on the basis of the achieved fertilization rate of more or less than 50% following the IVF method. This limit value was set to 90.85 % with 75 % sensitivity and 55.2 % specificity. Based on this we are able to separate semen samples suitable for the IVF method or ICSI method because we can anticipate the percentage of fertilized oocytes following the IVF method. The curve is statistically significant (*P* = 0.000) with the area below the curve (AUC^{ROC}) 0.721 (0.618–0.823) (95 % CI) (Figure 7).

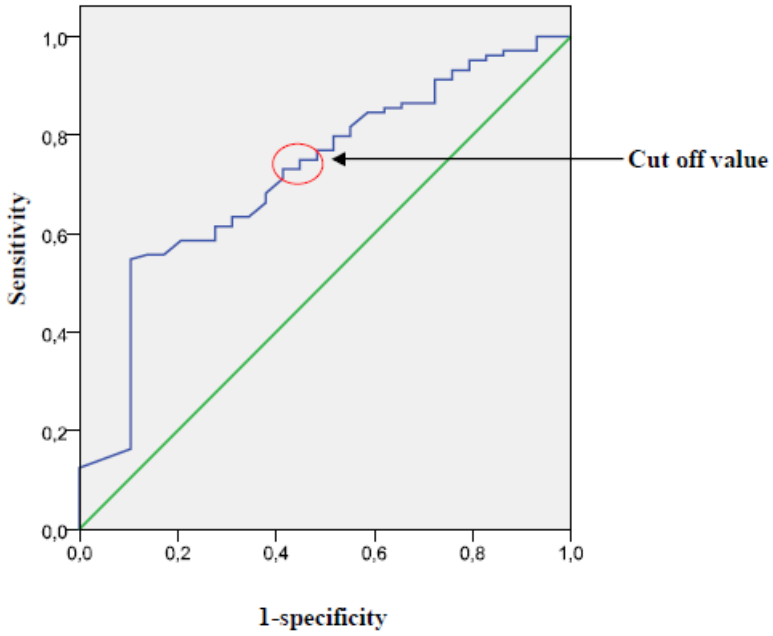

Figure 7. ROC curve representing the percentage of spermatozoa with hyaluronan-binding ability regarding the percentage of fertilized oocytes following the IVF method lower or higher than 50%.

Conclusion

We established in our study that the hyaluronan-binding ability analysed on a semen sample has s significant impact on the percentage of fertilized oocytes following the IVF method. Based on the result of the mentioned test we can estimate with certainty the fertilizing ability of spermatozoa in the IVF method and successfully differentiate the semen samples suitable for the IVF method from those less appropriate on account of the expected low share of fertilized oocytes (less than 50%).

WHY CAN THE HYALURONAN-BINDING ABILITY BE AFFECTED (CAUSES)?

Reactive Oxygen Species

Reactive oxygen species (ROS) are highly reactive free radicals. They are generated as undesirable side products of the oxidative energy metabolism.

From the biological perspective the following three compounds are especially important: hydrogen peroxide (H_2O_2), superoxide or superoxide anion radical ($O_2^{\bullet -}$) and hydroxyl radical ($OH^{\bullet -}$).

When the level of their concentration exceeds the limits tolerable and manageable for the cell, oxidative stress occurs and causes cell damage [56]. Free oxygen radicals with lipid peroxidation (oxidation of unsaturated fatty acids) damage the lipids in the cell membrane.

Spermatozoon's membrane is due its composition especially susceptible to ROS-induced damage. 50% of the spermatozoon's membrane is represented by the docosahexaenoic acid (DHA). DHA is a 22-carbon chain with six double bonds (polyunsaturated fatty acids). Oxidation of these fatty acids leads to a loss of fatty acids from the cell membrane structure, which causes instability and dysfunction of the cell membrane [57].

Therefore the membrane looses the fluidity, ability to maintain ion gradient and successful course of transport processes as well as the position of receptors in the membrane [58].

The spermatozoon is under different conditions exposed to excessive and unmanageable function of reactive oxygen species, which differ according to source and place of function:

- The spermatozoa travel after the completed spermatogenesis from the seminiferous tubules of the testes into the epididymis and remain there for days [59]. Among them are also immature or even dead spermatozoa which are the source of ROS.
- Banks et al. [60] concluded in his study that exposure of testes to a high temperature (42 °C) evokes an increased ROS concentration in the epididymis as a reaction to higher temperature. Epididymis at higher temperature is no longer an appropriate environment for spermatozoa, which is reflected in the increased share of immotile and dead spermatozoa in the epididymis [61, 62], which are afterwards an additional source of ROS.
- Leucocytospermia (presence of leukocytes in the semen, their concentration higher than 1 million/ml): peroxidase-positive leukocytes (poly-morphonuclear leukocytes and macrofagues stem from the prostate and seminal vesicles [63] are the source of ROS [64, 65]
- smoking: nicotine has been linked with the increased production of ROS [66-68]
- alcohol: ROS are generated in metabolic pathways for the conversion of ethanol [69]

It is clear from the above stated that a spermatozoon is inevitably exposed to the function of ROS which have a different source and the duration of the exposure varies. However, the consequence of their action is the dysfunction of the membrane, affected structure and loss of receptors for binding to hyaluronan.

Irregularities in the Course of Spermiogenesis

During spermiogenesis the spermatozoa develop from the round spermatids. During this process histones are discarded and replaced by protamines, a part of the nuclear, half of the mitochondria and most of the cytoplasm are discharged, an acrosome develops, receptors are built into the membrane for binding to zona pellucida and binding to hyaluronan [32]. If a spermatozoon from the semen is exposed to hyaluronic acid, we can notice that certain spermatozoa successfully bind to hyaluronic acid (have acquired during spermiogenesis the receptors for binding to hyaluronan) and some not. Analysing the characteristics of spermatozoa with binding ability led to the

conclusion that these are in fact spermatozoa which discharged excessive cytoplasm during spermiogenesis, successfully set their chromosomal statue during meiosis and do not have fragmented DNA which is known to largely develop during spermatogenesis [19, 37]. This basically means that in order to successfully develop the hyaluronan-binding ability it is necessary and important that all stages of spermatogenesis are progressing normally. The spermatozoa which have successfully completed the spermiogenesis and successfully bind to hyaluronan also share certain other characteristics, which explains the cause and effect link between the mentioned characteristics.

Huszar et al. established in one of his studies that the quantity of creatine kinases, which is normally a measure for the quantity of remaining cytoplasm, correlates with the fertilizing ability of spermatozoa *in vivo* or male fertility where a successful transformation of spermatozoa during spermiogenesis, including the acquisition of hyaluronan-binding ability, of key importance [70].

The quantity of HspA2p protein is also related to the fertilizing ability of spermatozoa. HspA2 is a part of sinaptonemal complex included into the intracellular transport of enzymes for DNA repair mechanisms, explaining why only those spermatozoa which have a normal chromosomal status and no fragmented DNA, have the hyaluronan-binding ability [71].

USE OF HYALURONAN INSTEAD OF POLYVINYLPYRROLIDONE

In the ICSI method it is required to immobilize the spermatozoon for a successful selection and intracytoplasmic injection. The most commonly used medium for this process is polyvinylpyrrolidone (PVP). It is a large (molecule weight 360 kDa) water-soluble, highly viscous polymer made from the monomer N-vinylpyrrolidone [72].

During the process of selection and injection, we expose the spermatozoon to PVP and at injection insert a small amount of it together with the spermatozoon into the cytoplasm of the oocyte [73]. Despite the frequent use of PVP, studies have shown that exposure of spermatozoa to PVP cause irreparable damage to the spermatozoa. PVP has a negative effect on the important structures of spermatozoon, such as acrosome, mitochondria, and plasma membrane [74]. After intracytoplasmic injection, PVP interrupts the

characteristic calcium oscillations, which are important for the fertilization process and even interrupts the decondensation of sperm chromatin [75].

Consequently, it affects the percentage of fertilized oocytes, embryo development [76-78] and embryo chromosome irregularities [79].

As PVP is a large polimer, it cannot be discharged from the oocytes and is not decomposable with cell enzymes and thus remains in the oocyte or embryo longer [80].

On account of the mentioned characteristics of PVP, an alternative and more physiological medium is increasingly used. Like PVP, this medium has to be highly viscous to successfully slow the spermatozoa, their attachment to the bottom of the dish and has no influence on the fertilization of the oocyte or further embryo development.

Hyaluronan is in addition to the stated characteristics, naturally present in the human organism and as part of the extracellular matrix of the cumulus cells directly participates in the fertilization process of the oocyte.

Unlike PVP, is decomposes inside the cell into monosaccharides in the usual biochemical manner and therefore represent a more physiological alternative to the normally used PVP.

Several studies confirm the successful transition from PVP to hyaluronan and successful outcome of the ICSI procedure [21, 22].

HYALURONAN IN EMBRYOTRANSFER MEDIUM

Hyaluronan can be found besides in cumulus cells also in cervical mucus, fallopian tube, uterus and follicular fluid [81]. The amount of hyaluronan in the endometrium changes during the menstrual cycle, an increased amount of hyaluronan in the endometrium is noticeable during the receptive phase of the endometrium [82] and the receptors for binding to hyaluronan on the surface of the human embryo through the entire pre-implantation phase [83]. They are the so-called CD44 receptors from the family of cadherins, by means of which the embryo is able to bind to hyaluronan. All this indicates the important role of hyaluronan in the pre-implantation process [84] where hyaluronan stimulates important intercellular interactions during the initial stages of the adhesion of the blastocyst to the endometrium [85, 86]. Several studies have been performed with the purpose to establish the effect of hyaluronan (presence in the embryotransfer medium, EmbryoGlue (Vitrolife, US)) of the success of embryo implantation. The studies compared the implantation rate and percentage of clinically confirmed pregnancies between groups where the

embryotransfer was carried out from a medium containing hyaluronan (EmbryoGlue) and from a medium without it. Several studies have confirmed a positive effect of using hyaluronan medium on the implantation of the embryo [24-26, 87].

A positive effect of hyaluronan on the percentage of clinical pregnancies was confirmed by less studies [25, 26, 87, 88], while the remaining could not confirm this effect [24, 89-91].

CONCLUSION

Ubiquitarian hyaluronic acid in the human body represents an important compound with great significance in reproductive medicine. The sperm's hyaluronan-binding ability is unique. By observing this binding we are able to estimate its fertilizing ability, recognize the causes of male infertility and contribute to a successful outcome of treating infertility.

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