A review of possible applications of polarization microscopy in IVF

Polarizasyon mikroskobunun IVF uygulamalarında muhtemel kullanım alanları

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Abstract

Polarization microscopy allows non-invasive imaging of highly ordered cellular structures which cannot be seen with conventional light microscopes. In IVF, the most frequently analyzed cell component is the meiotic spindle. It became clear that not only the presence of the meiotic apparatus is of prognostic potential, but also the intensity of the birefringence may provide valuable additional information concerning the implantation behavior of the resulting embryo or blastocyst. In addition, the retardance of the inner zona layer was found to be representative for oocyte quality. Last but not least, the male gamete has also been shown to have a birefringent capacity which allows for better selection of spermatozoa prior to ICSI. It can be summarized that polarization microscopy proved to be a reliable and useful tool in selection of both oocytes stemming from healthy follicles and spermatozoa with optimal spermatogenesis.

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Key words: Meiotic spindle, oocyte quality, polarization microscope, sperm selection, zona pellucida

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Özet

Polarizasyon mikroskobu, normal ışık mikroskopları ile görülemeyen hücresel yapıları görüntüleyen non invazif bir yöntemdir. IVF de en sık incelenen hücresel yapı mayotik iğciklerdir. Sadece maytik iğciklerin yoğunluğu değil, aynı zamanda çifte kırılım seviyeleri de implantasyon veya blastokiste gidiş potansiyelini göstermesi bakımından önemlidir. Ayrıca iç zona daki materuyaller de oosit kalitesini yansıtır. Aynı zamanda erkek gamette de bu çifte kırılım özelliği nedeniyle ICSI öncesi uygun spermatozoa seçimi mümkün hale gelir. Özetle, polarizasyon mikroskobu hem uygun oositin, hem de en uygun spermin seçiminde uygun bir araçtır. (J Turkish-German Gynecol Assoc 2009; 10: 104-8)

Anahtar kelimeler: Mayotik iğcikler, oosit kalitesi, polarizasyon mikroskobu, sperm seçimi, zona pellucida

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Introduction

By definition, polarization is a property of waves that describes the orientation of their oscillations. Birefringence, or double refraction, is the decomposition of a ray of light into two rays (a fast and a slow axis) when it passes through certain types of material (e.g. cellophane, calcite). In relation to the fast axis, the progression of the light along the slow axis is retarded. This effect may occur only if the structure of the material is anisotropic (directionally dependent). For biological material, this means that structures visible under polarized light have to be highly ordered (e.g. meiotic spindle, inner zona layer). In detail, when entering the birefringent material, light is split into two beams and oscillates on both optical axes in phase. During the passage through the material the two light beams are moving with different progressive speeds resulting in a phase shift. This phase shift (in nm) is called retardance and reflects the material's magnitude of birefringent power.

In the last decade, polarization microscopy was introduced in laboratories for analyzing cellular components of animal cells (1). Some 50 years ago polarized light was used to visualize the microtubule-dependent birefringence of the mitotic spindle (2). In living spindles, polarization microscopy also turned out to be a helpful tool (3). In modern times, polarization microscopy is any form of microscopy capable of detecting birefringent objects. It is usually performed with a polarizing element below the heated stage to produce plane polarized light and an analyzer that is set to give total extinction of the background and thus to detect any birefringence (4, 5). More recently, a new type of polarization microscope system was developed using liquid crystals to modulate the polarization state (6). With improvements in computer technology, this principle not only enabled real-time visualization of birefringent structures but also real-time calculation of polarization parameters (7).

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Polarization microscopy also attracted researchers in the field of assisted reproduction since on the one hand it is a non-invasive method, and on the other, it allows high-resolution imaging of birefringent structures with poor contrast, like the spindle apparatus which is not visible with conventional contrasting methods like Hoffmann-, DIC- or relief contrast. In addition, quantification of birefringence and analysis of the molecular orientation inside those structures is also possible.

Meiotic spindle

The first cellular organelle analyzed using polarized light is the meiotic spindle. Since spindles are highly dynamic with a relatively complex structure, it is evident that any disturbance in culture conditions will cause decay of the spindle microtubules. Thus, it is crucial to keep working conditions in the IVF lab constant (8). Most likely, shifts in temperature would cause damage to the spindle apparatus (9-11) regardless of whether this involves lethal warming of the oocyte (11) or detrimental cooling (12). Oxygen tension (13) and pH shifts (14) will have an additional impact on spindle condition. These physiological limitations have to be kept in mind when interpreting spindle data from polarization microscopy.

Spindle presence

The first scientists applying the innovative technique of polarization microscopy were Wang et al. (15). Probably due to methodical problems, these authors could detect meiotic spindles in less than two thirds (61.4%) of all mature gametes. Most probably oocytes were not screened for the presence of the spindle in different planes (16). However, their detection rate lies far beyond ($P<0.001$) that of any other study currently published (Table 1). Nevertheless, this investigation (15) provided the first evidence that the presence of a spindle apparatus is a positive prognostic marker of fertilization. Table 1 shows that several other study groups (16-19) were able to confirm this relationship.

Rienzi et al. (16) further suggested that the predictive power of birefringent spindle presence is associated with the degree of deviation between spindle and first polar body. They observed a 74% fertilization rate if spindle and polar body were more or less aligned and a reduced fertilization (50%) if the angle between them was >90 degrees. Others could not support this hypothesis (17).

The further fate of spindle positive gametes seems to be better compared to that of oocytes showing no spindle under polarized light. At least the cell number on day 3 of preimplantation development was superior (19). The possible correlation between spindle presence and the degree of fragmentation has been controversial (16, 17, 20, 21).

Although it is likely that the correlation between day 3 morphology and blastulation is limited, one study (19) revealed that eggs showing a meiotic spindle reached blastocyst stage (51.9%) significantly ($P<0.001$) more often than oocytes without a spindle (29.9%). This is in line with a Hungarian report indicating a relationship between spindle positivity and the occurrence of a clinical pregnancy (22).

Recently, a meta-analysis has been published dealing with the actual relationship between visualization of meiotic spindles in human oocytes and ICSI outcome (25). This review summarized results of 10 papers that fulfilled inclusion criteria. Interestingly, all analyzed parameters, such as fertilization rate ($P<0.0001$), zygote morphology ($P<0.003$), cleavage rate ($P<0.0001$), embryo quality ($P<0.003$), as well as rate of blastocyst formation ($P<0.0001$) were higher than in the spindle negative counterparts.

Recent findings of Montag et al. (26) have to be taken into account when discussing this data. These authors stressed the impact of the meiotic cell cycle on the presence of the spindle. Video sequences showed that during the transition from metaphase I to metaphase II the spindle apparatus completely disappears for approximately one hour. This supports the idea that in some oocytes the absence of the spindle is more likely an indicator of physiological progression through an important developmental stage of meiosis rather than a cellular disturbance. In view of the low fertilization rates of oocytes with absence of spindles as reported in the literature, the problem could simply be an incorrect timing of ICSI (26).

Table 1. Presence of the meiotic spindle and its association with fertilization rate

Authors	Spindle positive	Fertilization rate	
		spindle	none
Wang et al. (15)	327/533 (61.4)	202 (61.8)[a]	91 (44.2)[a]
Rienzi et al. (16)	484/532 (91.0)	362 (74.8)[d]	16 (33.3)[d]
Moon et al. (17)	523/626 (83.6)	444 (84.9)[c]	78 (75.7)[c]
Wang et al. (19)	1266/1544 (82.0)	879 (69.4)[b]	175 (62.9)[b]
Cooke et al. (20)	115/124 (92.7)	81 (70.4)	nda
Cohen et al. (21)	585/770 (76.0)	413 (70.6)[e]	115 (62.2)[e]
Konc et al. (22).	320/428 (74.8)	235 (73.4)	nda
Shen et al. (23)	739/897 (82.4)	676 (91.5)[f]	116 (73.4)[f]
Rama Raju et al. (24)	160/205 (78.1)	132 (82.5)[g]	14 (31.1)[g]

Values in parentheses are percentages; nda: no data available

[a, b, c, e, g] $p<0,05$

[d, f] $p<0,001$

Spindle quality and retardance

It has been demonstrated that microtubules are responsible for spindle birefringence and that spindle retardance is directly proportional to microtubule density (27). In practice, even single and bundled microtubules can be measured adequately (28). In other words, spindles composed of numerous highly ordered microtubules have a higher retardance than spindles with poor organization.

It is important to know that composition and structure may vary with female age (23, 29). However, in an average patient cohort quantitative assessment of light retardance may be of benefit in terms of the selection process for transfer (23). In detail, Shen et al. (23) used the pole-to-pole distance of the meiotic spindle (long axis) as well as the absolute magnitude of retardation to describe the quality of the gamete.

Those zygotes with a good pronuclear pattern 0B or 3 according to Montag and Van der Ven (30) derived from oocytes with a significantly ($P<0.05$) higher spindle retardance (1.72 nm) than those zygotes stemming from intermediate (1.53 nm) and bad patterns (1.52 nm). The retardance of oocytes that led to bad suspicious zygotes (uneven size of pronuclei, failure of pronuclear contact, 1 or 3 pronuclei) was worst (1.39 nm).

In the same study (23), spindle length was not related to pronuclear pattern; however, in the case of shortened spindles, the risk of pronuclear misalignment was significantly higher ($P<0.001$).

The magnitude of spindle retardance may also predict blastomere number on day 3 of preimplantation development (31) and blastocyst formation (24). The latter data set indicated that oocytes with a spindle retardance of more than 3 nm showed improved progression ($P<0.05$) to blastocyst stage (61%) as compared to oocytes with a retardance of 2-3 nm (25%) or less than 2 nm (14%). In addition, a correlation was found between spindle length and blastulation. If the barrel-shaped spindle was longer than 12 nm, survival to day 5 was significantly ($P<0.05$) better (45%) than in the groups with shorter spindles, e.g. 10-12 nm (34%) and <10 nm (20%).

Since spindle formation and retardance are prone to suboptimal culture conditions, measurement of the magnitude of light retardance may serve as a tool for quality maintenance in an IVF laboratory (32, 33). However, considering the highly dynamic structure of the spindle, its dependence on orientation and physiological conditions (7), the predictive value still remains to be clarified.

ICSI after spindle assessment

There is striking evidence that the position of the first polar body does not automatically indicate the actual site of the meiotic spindle (34, 35), mostly due to the manipulation process during denudation. Consequently, there was legitimate fear of damage to the spindle structure, if the spindle is dislocated and lies within the injection path. In the meantime, experience has shown that the meiotic spindle is a rather rigid structure that cannot be damaged by direct contact.

Thus, some authors (36) used polarization microscopy to perform spindle aligned ICSI (meiotic spindle 90 degrees from injection site). This new technique was compared with the usual polar body aligned ICSI. Interestingly, the number of blastomeres on day 2 ($P<0.05$) and morphology of the embryos were superior in the spindle-aligned group vs. the polar body-aligned cohort ($P<0.01$). Fertilization rate was not affected.

Others (37) questioned the usefulness of spindle imaging since spindle position assessment did not improve ICSI outcome. In fact, the best results in terms of fertilization and embryo quality were seen if the spindle was in or near the plane of injection (3, 4, 8 and 9 o'clock position).

The above mentioned fact that spindle formation is cell cycle dependent as well as the observation that not all oocytes showing a first polar body are at metaphase II (38), support the hypothesis that at least the timing of ICSI can be improved if the polarization microscope is used. In this respect, Cohen et al. (39) observed significantly more spindle positive eggs ≥38 hours post hCH administration (82%) compared to earlier induction (62%). Montag et al. (26) could demonstrate that a single observation could be inadequate and suggested repeating the analysis and/or postponing ICSI by 2-3 hours if the spindle was absent at the time of checking. In particular, ICSI involving the use of polarized light will bring benefit in patients having a limited number of gametes.

Zona pellucida imaging

Recently, it has been shown that the multilaminar structure of the zona pellucida can also be analyzed quantitatively using polarized light microscopy (40). Although considerable variation exists in the thickness of zona layers around individual eggs and between members of a cohort, it is evident that the inner zona layer is the most dominant part of the zona (23, 40). Since the inner layer of the zona is highly ordered it can clearly be depicted using polarized light. It has been reported that the birefringence of the inner zona is directly proportional to its thickness (23, 24, 40). It has also been shown that the mean difference in thickness between zonae from conception cycles ($11.3\pm1.4\mu$m) and failed ones ($9.4\pm1.7\mu$m) was around 1μm (23), a value being beyond the limit of provability for most systems designed for measuring cells. Using retardance measurement, however, prognostic power in terms of pregnancy was increased. In detail, Shen et al. (23) found an almost 30% higher mean light retardance in conception cycles as compared to non-conception cycles, indicating that some stimulated cycles yield oocytes of reduced quality. Recently, subjective zona imaging of unfertilized MII-oocytes was successfully used as the primary selection criterion for embryo transfer (41), indicating the predictive power of zona pellucida characteristics for the further fate of the concepti.

There are two retrospective studies suggesting a relationship between zona birefringence (inner layer) and preimplantation development. Montag et al. (42) noted a higher rate of good quality embryos on day 3 (but not on day 2) in the oocyte group with high zona birefringence (41.7%) as compared to the cohort with low birefringence (24.4%). An Indian group (24) observed a difference in progression to blastocyst stage. If the zona inner layer retardance was >3nm, the blastulation rate was 60.9% as compared to 14.1%, if retardance was lower than 2nm.

Since all data dealing with zona imaging were of a retrospective character, a prospective study was set up in order to determine the potential of this rather new technology (43). It could be shown that the only parameter affected was the blastulation rate, which was increased if the retardance of the inner zona layer (figure 1) was high ($P<0.05$).

To summarize, all these results indicate that zona pellucida appearance could function as a marker of optimal folliculogenesis and/or oocyte maturation since it is well accepted that the developmental fate of an embryo is largely dictated by the quality of the oocyte, which in turn reflects the follicular milieu.

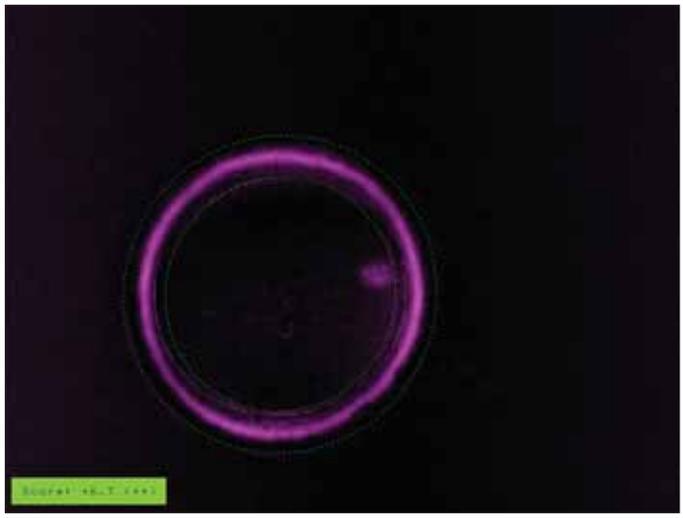

Figure 1. Automatic user-independent zona pellucida imaging of mature oocyte

Sperm birefringence

As was demonstrated, polarization microscopy can be a powerful tool in selecting gametes by combining information from spindle detection and zona pellucida imaging. With respect to gamete selection, the male gamete is frequently underestimated. Baccetti and co-workers (44) proposed an innovative approach, namely using polarized light for sperm selection. This technique is based on the birefringence characteristics of spermatozoa due to their anisotropic properties of the cytoplasmic texture. Obviously, in a mature sperm nucleus, there is a strong intrinsic birefringence associated with nucleoprotein filaments that are ordered in rods and longitudinally oriented. The same will hold for the acrosomal complex, since subacrosomal protein filaments cause a similar type of birefringence. Even the tail is in part birefringent due to the microtubular organization (44).

Gianaroli et al. (45) were the first to apply this new method in 112 couples and compare embryo development and implantation with that of a patient group (n=119) subjected to routine ICSI. Their criterion for sperm selection was the presence of birefringence in the sperm head, regardless of whether it was associated with the nucleus or the acrosome. For practical reasons sperms were not screened for tail birefringence.

Interestingly, the proportion of birefringent spermatozoa was positively correlated with concentration, progressive motility and vitality (45). Fertilization rate and cleavage behavior did not differ between polarized and routine ICSI groups; however, more ($P<0.05$) excellent embryos were seen with the new approach (33% vs. 20%). Accordingly, the implantation potential of embryos deriving from birefringent sperms was higher (19%) than that observed in the conventional treatment group (11%). Pregnancies occurred at a similar rate (31% vs. 21%); however, due to a higher incidence of missed abortions, the clinical pregnancy rate differed significantly, e.g. 23% in the modified ICSI group and 11% in the routine one.

Summary

It can be summarized that polarization microscopy proved to be a reliable and useful tool in the selection of both oocytes stemming from healthy follicles and spermatozoa with optimal spermatogenesis.

In oocyte spindle detection it became clear that not only the presence of the meiotic apparatus is of prognostic potential but much more, the intensity of the birefringence may provide additional valuable information concerning the implantation behavior of the resulting embryo or blastocyst. In addition, the shape and length of the spindle image may be conclusive in terms of spindle quality, e.g. number of fibers.

In oocyte selection, zona pellucida imaging brought additional selective information; however, at the beginning, values for the zona were calculated based on a rather small number of measuring points. This did not address oocytes with inhomogeneous zonae. The latest achievement of an automatic detection mode for the inner layer of the zona pellucida uses more than 100 measurements and is definitely an improvement in IVF labs. It will help to further minimize the time necessary for analysis which is a prerequisite for optimal in vitro growth.

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