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Clinical Assisted Reproduction

Influence of Polarization Effects in Ooplasma and Pronuclei on Embryo Quality and Implantation in an IVF Program

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***Purpose*:** The presence of a clear half-moon-like zone of cytoplasm in oocytes is called "halo effect." The prognostic value of this effect is not yet determined. Aligned nucleoli in pronuclei (PN) represent a further polarization phenomenon and a marker for implantation potential. Aim of the prospective study was to evaluate the influence of the halo effect on IVF outcome and to compare the results with observed polarization in PN.
***Methods*:** A total of 374 cycles with embryonic transfer were analyzed regarding halo effect and pattern of nucleoli. The oocytes were single-cultured to observe the following embryo quality of each PN stage.
***Results*:** Cycles with halo-positive oocytes showed a significant higher pregnancy rate (44.0% vs. 31.1%; $p < 0.05$). Furthermore, higher pregnancy rates in cycles with polarized nucleoli were observed. Polarized PN resulted in a significant lower fragmentation and higher cleavage rate of embryos. The fragmentation rate was significantly lower in halo+ oocytes, but the cleavage rate was not influenced.
***Conclusions*:** The results indicate that the presence of a polarized zone of human fertilized oocytes can be a useful indicator for good oocyte quality. Since the origin of ooplasmic polarization seems to be a different process compared with the alignment of nucleoli, the observation will give additional predictive information about the implantation potential.

KEY WORDS: Embryo quality; halo effect; oocyte; polarization; pronuclei.

INTRODUCTION

The generation of a new organism in vertebrates is a highly complex process, and the regulation is not completely understood. In each species including human, the development of an embryonic axis could be observed (1–4). The origin of this early differentiation is due to a polarization, which seems to exist already on the level of the gametes (5,6). Biochemical polarization of the oocyte and early embryonic stage could be shown by detecting marker substances like leptin or several growth factors (7). Polarization seems to be an important factor for development and implantation. In normal fertilized human oocytes at two-PN (two-pronuclei) stage a polarization can be observed by light microscopy. The presence of a clear half-moon-like zone of cytoplasm in one pole of the cell is called "halo effect." The origin of this area is due to the displacement of mitochondria and further cell organelles to other regions of the oocyte. Microtubules are known to possess the ability to move intracytoplasmic organelles in a physiological way (8). However, the prognostic value of this effect is not yet determined.

Because of the German embryo protection law (Embryonenschutzgesetz), the selection of a

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maximum of three embryos has to be done on the level of PN stages. Selection criteria are the exclusion of oocytes with morphologic irregular pattern, which are able to influence the outcome in IVF (9). The development of a PN score based on substructures of the PN was developed in the last years by Scott (10) and Tesarik (11). The presence of polarized nucleoli on the fusion side of the PN seems to be a good-quality criterion for fertilized human oocytes. However, the clinical benefit is not yet established. The polarization of cytoplasm in human oocytes might be a third criterion on the level of PN stages. Our intention was to examine a possible correlation between a detectable halo effect on the level of PN stages and the outcome for the patients, in comparison with polarized PN and embryo quality in an IVF program.

MATERIAL AND METHODS

In a prospective study of 374 patients with embryonic transfer in an IVF program over 18 months, the presence of oocytes with or without halo effect was investigated, and the data were compared with the outcome of the IVF/ICSI. MESA-TESE-patients were excluded from this study. All patients were stimulated with either Flare-up GnRH-Analogon/hMG therapy (Supreccur®; Hoechst, Germany; and Menogon®, Ferring, Germany), or downregulation occurred with Leuprorelin (Enantone Gyn®, Takeda, Japan) and stimulation with recombined FSH (Gonal F®, Serono, Germany). The ultrasound-guided follicle puncture took place transvaginally. The insemination occurred conventional (IVF) with 50,000–100,000 spermatozoa/mL 4/5 h after oocyte retrieval or by microinjection of one spermatozoon into the oocyte (ICSI). If fertilization took place, every PN stage was cultured in an 5 μL droplet of IVF medium (MediCult, Denmark) until embryo transfer, covered with mineral oil (Sigma-Aldrich, Germany). Therefore, the embryo quality of each oocyte could be observed individually. Not more than three embryos (because of the German law) were transferred transvaginally 2 or 3 days after puncture. No selection on the level of cleaved embryos was possible. To evaluate the embryo quality, we defined a score system. We classified the embryos on Day 2 into four groups according to Steer *et al.* (12): A = no fragmentation, equal blastomeres; B = small fragmentation (<20%); C = 20–50% fragmentation; and D = high fragmentation (>50%). The number of blastomeres and the fragmentation grade (A = 4; B = 3; C = 2; D = 1) were added to a score. Pregnancy was defined as a serum β-hCG-level >30 mLU/mL 2 weeks after puncture, combined with the detection of a gestational sac.

To observe the oocytes at PN stage, we use an inverse microscope (Nikon, Japan) and a micromanipulator (Narishige, Japan) to examine the cells from every position. Each oocyte was turned with a micropipette (Humagen, USA). Halo effect was assumed, when the clear cytoplasmic area of the oocyte was not wider than 180° of the cell circumference and exactly distinguishable from the darker granular structure of the rest of the cytoplasm (Fig. 1). Furthermore, the clear cytoplasmic area has to be restricted to one cell pole, which can be checked by turning the oocyte with a micropipette.

The PN score was evaluated in the same way. Observations were done, when both PN were situated in the same plane. Good-quality-PN stages (al+) were defined according to Tesarik as oocytes with moderate number of nucleoli (4–8), which are aligned in both PN on the fusion side (Fig. 2). In the study of Tesarik, this pattern was called Z0B. Other PN stages with numbers of nucleoli <4 or >8 or without alignment in both PN were calculated as poor-quality-PN score (al−). The selection of PN stages was performed not only in regard to PN score or halo effect, since other criteria such as morphological pattern were able to influence the implantation rate. Oocytes with vacuoles, irregular shape, or dark granulation were, if possible, not selected for embryo transfer. The results in all groups were compared with the IVF outcome.

RESULTS

Halo Effect

A total number of 374 cycles were analyzed. In 115 IVF patients with oocytes missing any halo effect (halo−) the pregnancy rate was significantly lower than that in 259 patients who had at least one oocyte with polarization (halo+). A total number of 106 pregnancies were achieved in the halo+ group (40.9%), whereas in the halo− group only 25 women became pregnant (21.7%). The differences in pregnancy rates were highly significant ($p < 0.001$). We could demonstrate that age of the women, number of punctured oocytes, maturation stage of the oocytes, and embryo score were not significantly different in both groups (Table I). The indications for the couples were similar in the groups: tubal factors 33.6 and 33.3%; endometriosis 8.0 and 11.9%; polycystic ovaries 30.0 and 30.4%; andrological diseases 64.0 and 67.4%. In most cases, hMG/flare-up was

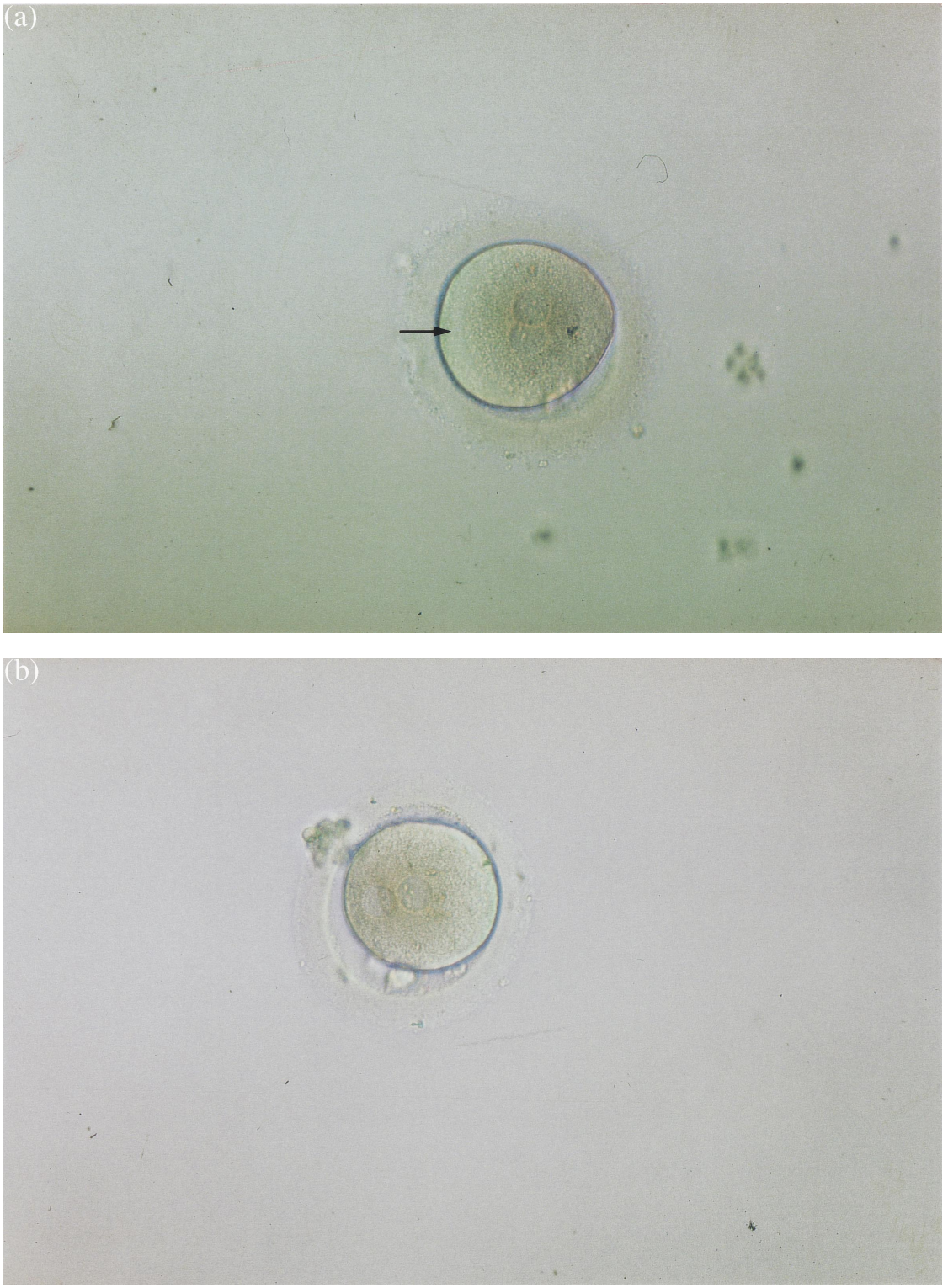

Fig. 1. (a) 2-PN stage with clear visible halo effect (arrow) in one pole of the oocyte. (b) Normal fertilized oocyte without halo effect. (c) 2-PN stage with a clear zone of cytoplasm around the center (arrows). This area is not polarized (>180°) and excluded from the study. To analyze the real conditions of the oocyte, the cells have to be turned to all positions with a micromanipulator.

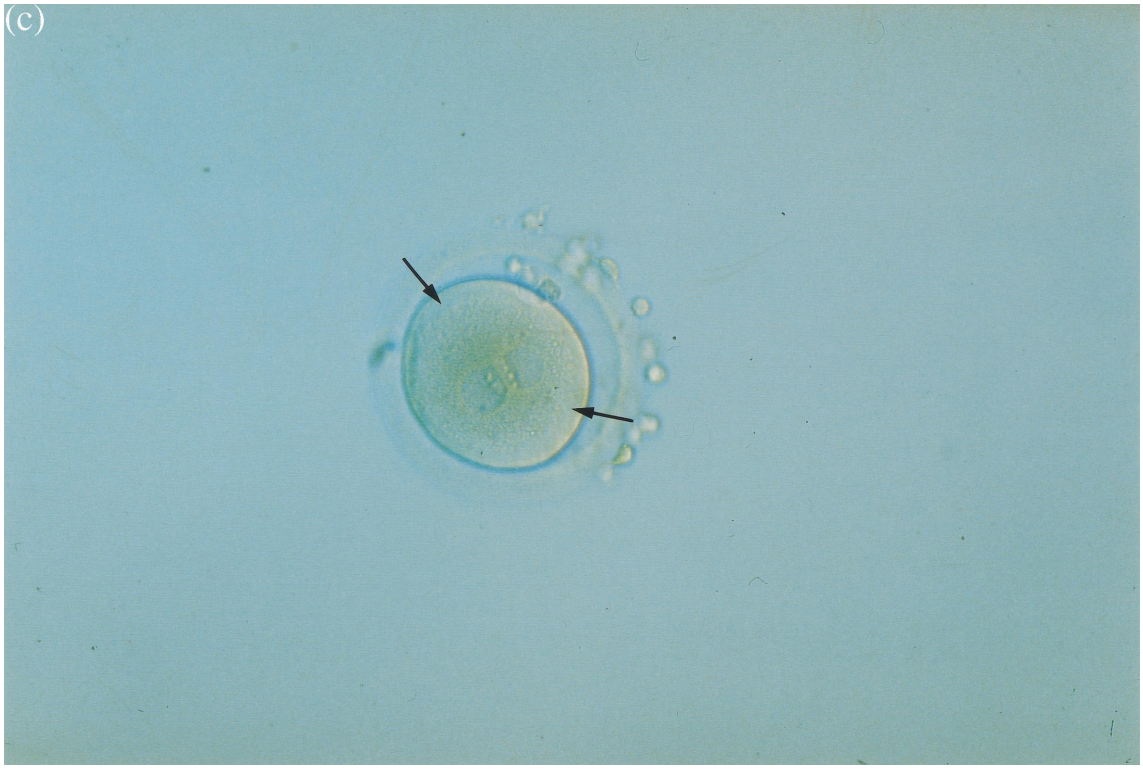

Fig. 1. (*Continued*)

used as stimulation protocol. The average number of administered hMG-ampules was not statistically different in each group (Table I). Nevertheless, the group of cycles missing any halo effect showed a significant lower fertilization rate (73.3% in the halo+ group vs. 66.5% in the halo− group; $p < 0.001$) following a reduced number of embryos transferred (2.68 embryos/transfer in the halo+ group vs. 2.27 embryos/transfer in the halo− group; $p < 0.05$). This might be the reason for a decreased pregnancy rate. To overcome the influence of fertilization rate and embryo number, we analyzed in a further step only those cycles with three transferred embryos. Although the pregnancy rate in the halo+ group was significantly higher (44.0% in halo+ vs. 28.3% in halo−; $p < 0.05$), no differences were seen on the other parameters. In both groups the age of the female patients, number of retrieved oocytes, maturation stage, fertilization rate, and number of embryos per transfer were similar. The embryo quality was comparable in both groups (score: 6.0 vs. 6.1; ratio of two-cell stages/four-cell stages: 1.11:1.10). However, the number of fragmented embryos (>20% of the volume filled with vesicles; quality C and D) was lower in the group with halo effect (23.8% vs. 30.6%; $p < 0.05$). By analyzing only those oocytes which were selected for embryo transfer and not the total number of PN stages, we obtained comparable results in the groups with and without halo effect, respectively (pregnancy rate in cases of three transferred embryos: 44.4% vs. 32.2%; $n = 233$ and 113; $p < 0.001$). There were no significant differences in outcome if the halo+ oocytes were selected for transfer or not.

The individual culture of each oocyte allowed the analysis of embryo quality in regard to the halo effect (Table III). A significant lower fragmentation rate was obtained in the halo+ group (23.8% vs. 30.6% embryos in quality C and D with more than 20% fragmentation; $p < 0.05$). No differences could be seen in the cleavage rate (38.1% vs. 39.9%, four-cell stages 42 h after insemination).

PN Score

A total number of 266 cycles with three transferred embryos were analyzed for good-quality-PN stages with aligned nucleoli (al+) (Table II). In 189 cycles, at least one al+ oocyte could be seen. The pregnancy

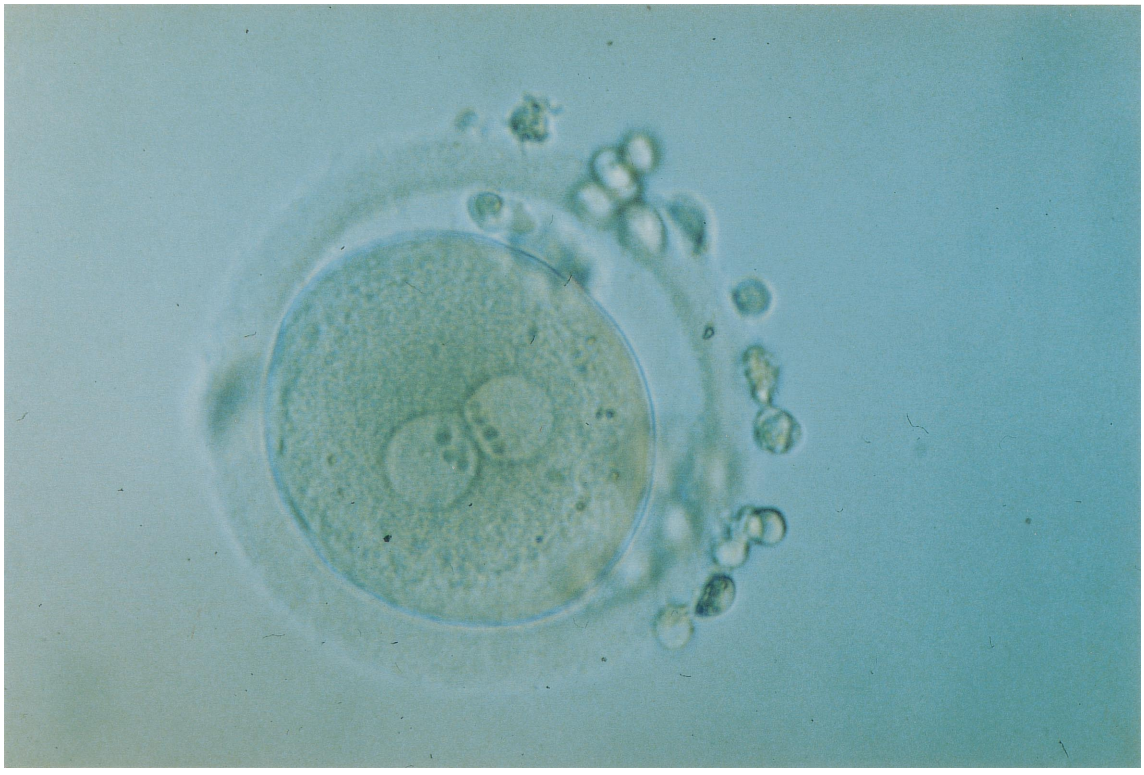

Fig. 2. Fertilized oocyte with aligned nucleoli in both PN. The small bodies are polarized on the fusion front of the PN.

rate in this group was higher in comparison to al− cycles (41.3% vs. 37.7%), but the results do not reach statistical significance. No differences were seen in the female age. By analyzing those cycles, in which al+ oocytes were selected for embryo transfer, we achieved comparable results (pregnancy rate 41.6% vs. 37.7% in al− cycles). Significant differences were detected by analyzing the embryo quality resulted from each PN stage (Table III). In the group of al+ oocytes, the fragmentation rate was lower (23.1%

Table I. Pregnancy Rates of Cycles Including Oocytes With Halo Effect (Halo+) and Without This Effect (halo−)

	Total number of cycles			Cycles with 3 transferred embryos		
	Halo+	Halo−	Significance	Halo+	Halo−	Significance
Cycles	259	115		207	67	
Pregnancies	106 (40.9%)	25 (21.7%)	$p < 0.001$	91 (44.0%)	19 (28.3%)	$p < 0.05$
Age of the women	32.9	34.1	ns	32.5	33.3	ns
hMG/flare-up cycles	249	115		193	61	
Average hMG-ampules/cycle	26.0	28.2	ns	25.5	24.9	ns
Oocytes (per cycle)	2.176 (8.2/C.)	853 (7.0/C.)	ns	1.872 (9.1/C.)	583 (9.0/C.)	ns
Metaphasis II	1.958 (89.9%)	735 (86.2%)	ns	1.699 (90.7%)	518 (88.8%)	ns
Fertilization	1.601 (80.8%)	572 (74.2%)	$p < 0.001$	1.421 (82.9%)	426 (79.6%)	ns
Embryo per transfer	2.7	2.3	$p < 0.05$	3.0	3.0	ns
Embryo score	6.0	5.8	ns	6.1	6.0	ns
Ratio: 2 cells/4 cells	1.11	1.10	ns	0.91	0.86	ns
>20% fragmented embryos	23.8%	30.6%	$p < 0.05$	25.4%	34.7%	$p < 0.05$

Note. The differences in pregnancy rates were highly significant. No significant differences were observed in mean age of the women, the number of punctured oocytes, maturation state, and resulting embryo score. Moreover, the number of administered ampules of hMG, which was used in most cases, was equal in each group. Nevertheless, the halo+ group showed a significant higher fertilization rate which leads to a higher pregnancy rate in the total group of cycles. In cases of three transferred embryos a significant difference in pregnancy rate between both groups could be detected, although other parameters, which are known to influence the implantation, showed no differences.

Table II. Comparison of Pregnancy Rate in Cycles Undergoing Transfer of Three Embryos Regarding Presence (Halo+) or Absence (Halo−) of Oocytes With Polarized Ooplasm, PN Stages With Polarized Nucleoli (al+) or not (al−), and Embryo Quality, Defined as 4-Cell Stage With Less Than 20% Fragmentation 42 h After Insemination

	Cycles	Pregnancies	Female age
Halo−*	67	19 (28.3)	33.3
Halo+*	207	91 (44.0)	32.5
Halo− (selected)**	87	28 (32.2)	33.2
Halo+ (selected)**	205	91 (44.4)	32.7
al−	77	29 (37.7)	32.9
al+	189	78 (41.3)	32.7
al− (selected)	98	37 (37.7)	32.7
al+ (selected)	166	69 (41.6)	32.8
0 4-cell stage A/B***	141	39 (27.6)	32.3
>0 4-cell stage A/B***	493	211 (42.8)	33.2

Note. The comparison was distinguished between the total number of observed halo+/al+ and the restricted number of selected oocytes for embryo transfer. No differences could be seen in the female age. Values in parentheses are in percentage.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

vs. 29.5% embryos C and D; $p < 0.05$) than that in oocytes missing polarized nucleoli. Furthermore, the cleavage rate was higher in the al+ group (39.5% vs. 47.7%, four-cell stages 42 h after insemination; $p < 0.05$).

The number of cycles with three embryos was too low to obtain significant data with "mixed quality criteria." Nevertheless, the pregnancy rates were higher in cycles of oocytes with halo+ and al+ in combination: halo+/al+, 57/127 (46.0%); halo−/al−, 7/23 (30.4%). When >1 al+ and >1 halo+ oocytes were present, the pregnancy rate increased even to 55.3% ($n = 38$).

DISCUSSION

Polarization of oocytes and early embryos is a crucial point in the development of a mammalian organism. The origin of this phenomenon is not completely understood. There seems to be no preferred location for sperm entry in human oocytes (13,14) which could be the starting point for a polarized development. However, the formation of different compartments on the level of fertilized eggs can be seen by measuring concentration of cytoplasmic factors such as leptin or STAT (7), or directly by observation of a clear, half-moon-like area on one pole of the oocyte (halo effect). The origin of such clear cytoplasmic zone seems to be an active movement of mitochondria and other cell organelles in the direction of the PN and spindle (15). Furthermore, this polarized area is able to rotate relatively to the oolemma (5). Ooplasmic rotation seems to start at sperm entry and to be regulated by tubulin filaments of the sperm centrosome and aster. The unsymmetrical distribution in the cytoplasm of the oocyte leads to a polarization of the early preimplantation embryo already after the first cell divisions. It is obvious that fragmentation on embryos causes a reduction of the polarized distribution, since amounts of cytoplasmic volume tied off into small vesicles are lost for the embryo. This could be one explanation for the decreased pregnancy rate in an IVF program after transfer of fragmented embryos (16). Fragmented preimplantation embryos are considered as poor quality embryos. The fragmentation grade depends on various parameters such as culture conditions and oocyte quality (12). The quality of female gametes depends on endocrine environment and ovarian conditions. If there is a relationship between oocyte quality, oocyte polarization, and embryo quality, observations of polarization of fertilized human oocytes may be a useful tool to predict the implantation potential in the IVF cycle and may give information about the oocyte quality.

In a prospective study we demonstrated a significant positive correlation between the presence

Table III. Embryo Quality Resulted From Oocytes Regarding Halo Effect (Halo+/Halo−) or Aligned Nucleoli (al+/al−) in Single Culture

	>20% fragmentation		4-cell stage after 42 h		Poor quality (both)	
	n	%	*n*	%	*n*	%
Halo+	483	23.8	464	38.1	165	69.4
Halo−	584	30.6	643	39.9	177	64.9
Significance	$p < 0.05$		ns		ns	
Al+	253	23.1	156	47.7	119	61.4
Al−	527	29.5	308	39.5	213	75.3
Significance	$p < 0.05$		$p < 0.05$		$p < 0.01$	

Note. Halo+ oocytes showed a significant lower fragmentation rate and no differences in cleavage rate, whereas good-quality-PN score (al+) led to higher embryo quality concerning both fragmentation and cleavage rate.

of oocytes with halo effect and the pregnancy rate, although other parameters able to influence the implantation were comparable. The results were similar in cases where the maximum of three embryos could be transferred. The examination of this patient group and the exclusion of cycles with lower number of embryos or fertilization rates was necessary, since the patients without halo effect showed a lower fertilization rate and therefore lower number of transferred embryos which could be responsible for the lower implantation rates. By analyzing only those cases with three embryos, we obtained results which are able to demonstrate the clinical importance of the halo effect. Since the number of retrieved oocyte, maturation grade, fertilization rate, and number of transferred embryos were similar in both groups, the polarization of the ooplasm seems to be an independent factor able to influence the implantation. However, no additional increased pregnancy rates were obtained by selection of polarized oocytes on the level of PN stages. This might be an indication that the halo effect could be rather a criterion for the quality of an oocyte cohort than that of an individual human oocyte.

During the last years other microscopic examinations were performed to determine the developmental potential of fertilized human oocytes. Scott (10) and Tesarik (11) defined a PN-score system, classifying the number and orientation of nucleoli in the PN. Good-quality-PN stages show a moderate number of about five small nucleoli bodies which should be aligned on the fusion side of both PN. There is evidence that this Z0B-called pattern (Tesarik) leads to higher pregnancy rates, whereas the influence of other pattern is low (17). Although a correlation between high PN scores and pregnancy rates could be demonstrated, the influence of cytoplasmic factors were not included.

Our data demonstrates a higher pregnancy rate by PN stages with aligned nucleoli, although the results could not reach statistical significance. Nevertheless, the significant higher embryo quality in embryos derived from al+ oocytes is leading to the presumption that the benefit of selecting al+ oocytes can be demonstrated with higher cycle numbers.

Oocytes with halo effect showed a significant lower fragmentation rate of the resulting embryos but not increased cleaving rates, whereas oocytes with polarized nucleoli (al+) had lower fragmentation rate and a higher cleavage rate. The predictive value of the halo effect bases on cytoplasmic quality which might influence the implantation by the development of a polarization axis of the embryo. This axis can be disturbed by fragmentation in early cleavage stages. On the other side polarized nucleoli in PN stages represent good-quality oocytes on the level of nucleus and the genome. This might be an explanation for reduced cleavage rate in oocytes with a poorer PN-score, which could not be observed in the halo− groups. However, the difference between poor and good quality was higher in the halo group than that in the PN-score group. Moreover, the pregnancy rates in cycles with and without halo+ oocytes were comparable with the results of cycles with good embryo quality (at least one four-cell stage with less than 20% fragmentation 42 h after insemination). It is well known that the selection of good-quality embryos from Day 2 to Day 5 leads to a significant higher pregnancy rate. However, the selection of halo+ oocytes seems to have no benefit. Further studies with a higher number of cycles are necessary to elucidate a possible influence of selection of halo+ oocytes on the implantation rate. At present, examinations of polarized ooplasm possess a highly predictive value, it seems to be a sufficient tool for selecting PN stages in countries such as Switzerland or Germany where embryo selection is not allowed.

CONCLUSION

The potential of various ooplasmic patterns seen with light microscope prior to insemination has been demonstrated elsewhere. Several studies showed a correlation between PN score and implantation. The halo effect seems to be a third quality criterion for the developmental potential of human oocytes on the level of PN stages. Patients with oocytes showing halo effect had significant higher pregnancy rates. Halo effect allows a prediction of the implantation potential independent of indications like endometriosis or polycystic ovarian syndrome, which are known to influence oocyte quality. Furthermore, a higher pregnancy rate was observed in cycles with PN stages with aligned nucleoli, although the differences were lower than in the halo− group. However, no further benefit could be observed by selection of polarized oocytes from the total pool of PN stages.

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