

Influence of oocytes and spermatozoa on early embryonic development

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Objective: To evaluate the effect of oocytes and spermatozoa on early embryonic development.

Design: Retrospective study.

Setting: Infertility Clinic, the Family Federation of Finland.

Patient(s): Fifty-nine oocyte donation cycles with oocytes shared among 118 recipient couples.

Intervention(s): Culture of all fertilized oocytes.

Main Outcome Measure(s): Standard sperm (concentration, progressive motility, and morphology according to Tygerberg strict criteria) and embryo (morphology and cleavage stage) characteristics.

Result(s): A marked effect of the oocyte on both embryo morphology and blastomere cleavage rate was demonstrated. In addition, a significant sperm effect on blastomere cleavage rate was found. Sperm morphology as determined according to strict criteria rather than sperm count or progressive motility was positively associated with the blastomere cleavage rate. None of the measured sperm characteristics influenced embryo morphology.

Conclusion(s): Embryo morphology, i.e., fragmentation and blastomere uniformity, are predominantly determined by oocyte quality, whereas both the oocyte and spermatozoa influence the blastomere cleavage rate. (Fertil Steril® 2002;78:1082–7. ©2002 by American Society for Reproductive Medicine.)

Key Words: In vitro fertilization, embryo quality, oocyte, spermatozoa

Embryo quality is one of the most important factors determining the success of IVF-ET (1–3). Several studies have indicated a close relationship between the oocyte and embryo quality (4–7), although the contribution of spermatozoa to early embryo development has been less clear (8). Some investigators have demonstrated a significant effect of spermatozoa on both embryo morphology and the blastomere cleavage rate (9, 10), while others have failed to show any effect in IVF cycles using a couple's own gametes (11, 12). Since probably both the oocyte (4–7) and the sperm (9, 10, 13–16) quality influence embryo development, an analysis of these factors individually in a routine IVF program is not appropriate.

To analyze the possible role of spermatozoa in early embryonic development, oocytes from a single source should be inseminated with different sperm samples. In practice, this has been done in the oocyte donation (OD) program at the Infertility Clinic of the Family

Federation of Finland, Helsinki. Because of the constant shortage of anonymous egg donors, oocytes from a single donor were divided between two recipient couples so that both of them received an approximately equal number of oocytes. Then these two groups of oocytes originating from the same oocyte donor were inseminated by two different sperm samples. Since the start of our OD program in 1991, the record keeping included detailed tracking of all sperm and embryo characteristics as well as pregnancy outcome. We have retrospectively analyzed all OD cases in which oocytes have been divided between two recipient couples to investigate the individual role of oocytes and spermatozoa during early embryonic development.

MATERIALS AND METHODS

A total of 59 OD and 118 recipient cycles performed in the Infertility Clinic of the Family Federation of Finland during 1992–2001 were

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studied. The oocyte donors were unpaid volunteers <37 years of age. All oocytes were fertilized using normal insemination. The ovarian stimulation regimen, oocyte collection, and luteal support remained basically unchanged during this time and have been extensively discussed elsewhere (17). Briefly, the donors underwent pituitary down-regulation with a GnRH-a commenced in the midluteal phase of the previous menstrual cycle. During 1992–1996, after suppression was achieved, ovarian stimulation was performed using either hMG or highly purified FSH. Recombinant FSH was mostly used for ovarian stimulation in 1997–2001.

After oocyte collection, the quality of cumulus-oocyte complexes (COCs) were evaluated and COCs were randomly divided between two recipient couples so that both of them received an approximately equal number of oocytes. COCs were classified either as having expanded or compact cumulus and corona. The assessment of oocyte quality by light microscopy alone has its limitations as many biochemical and genetic properties of oocytes cannot be evaluated. Nevertheless, it has been suggested that the expanded cumulus and radiant corona indicate mature and good quality oocytes, while compact cumulus and corona characterize immature oocytes (18). Therefore, special care was taken to ensure that both recipient couples received an equal number of oocytes with well-expanded cumulus.

Patients underwent a semen analysis for evaluation of standard sperm characteristics such as concentration, motility, and morphology. Sperm concentration was determined with the use of a Makler counting chamber (Sefi Medical Instruments, Haifa, Israel). Motility was expressed as the percentage of progressively motile spermatozoa, and the mean speed or motility of individual sperm cells was graded according to the World Health Organization recommendations (grades A, B, C, and D) (19). Morphology was evaluated on air-dried smears, fixed and stained by a modified Papanicolaou stain (Spermac; Fertipro, Beernum, Belgium) using Tygerberg strict criteria (20). At least 200 cells were independently examined by two technicians, and the mean of these two evaluations was used in the present study. All patients were divided into three groups according to the proportion of morphologically normal sperms (20). Patients in the first group had <4% morphologically normal sperm cells; patients in the second group had 4%–14% normal sperm cells; and patients in the third group had >14% normal sperm cells.

The semen sample was prepared by a 45%–90% discontinuous gradient method using Percoll (Pharmacia, Uppsala, Sweden) or PureSperm (Nidac International AB, Gothenburg, Sweden), and centrifuged at 550 *g* for 20 minutes. The pellets were collected from the bottom of the 90% layer, washed once with Universal-IVF medium (Medicult, Copenhagen, Denmark), and resuspended. After semen preparation, the concentration and motility of sperm cells were estimated. All oocytes were inseminated 5–6 hours after

retrieval with approximately 10⁵ progressively (grades A and B) motile spermatozoa in 1 mL Universal-IVF medium in Falcon (Becton Dickinson, San Jose, CA) single-well dishes with up to five oocytes per dish.

The oocyte maturity, according to nuclear maturation, and fertilization were determined 18 hours after insemination. The embryo quality, i.e., the number of blastomeres and the embryo morphology, was assessed on the second day (44–46 hours) after insemination. Embryo morphology was scored according to commonly used morphological criteria as follows: grade 1: no fragments and equal blastomeres; grade 2: <20% fragmentation; grade 3: unequal blastomeres and/or 20%–50% fragmentation; and grade 4: >50% fragmentation (21). All embryo and sperm parameters were recorded for each patient and used later for statistical analysis.

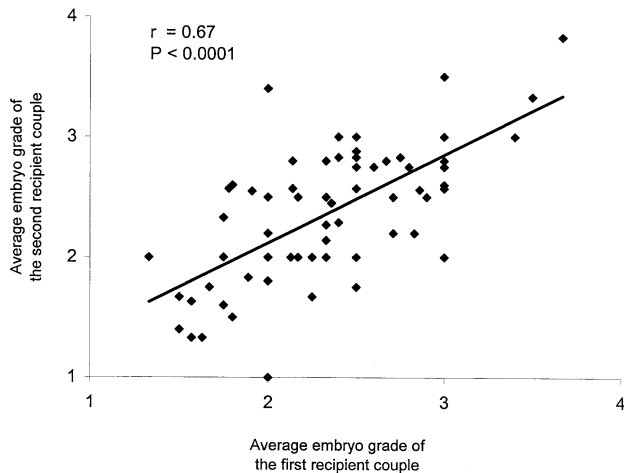
A maximum of two embryos were selected for placement into the uterus. The decision whether to transfer one or two embryos was made based on the patient's infertility history, previous treatments, age, and quality of embryos achieved. In some patients, however, all the embryos were cryopreserved on day 2 after insemination to provide better synchronization of the uterine endometrium and embryonic development in subsequent transfers of frozen-thawed embryos.

For evaluation of individual effects of oocytes and sperm cells on embryo quality, two alternative approaches were used. At first, the effect of the oocyte was ascertained by Pearson's correlation analysis using SPSS release 10.1.0. (SPSS, Inc., Chicago). For this purpose, the average blastomere number and embryo grade were calculated for both recipient couples of the same oocyte donor. Subsequently, the correlations between the average embryo characteristics of the first recipient couple and those of the second recipient couple were estimated.

The investigation of all putative factors that could influence the embryo quality was carried out using analysis of variance (ANOVA) as implemented in the mixed procedure of the SAS system, release 8.1 (22). This approach assumes that the variability in examined embryos results from the variability of oocytes, sperm samples, and some other unknown (residual) factors such as random differences between oocytes of the same donor. The test estimates the contribution of each of the studied factors to the overall embryo variability and relates each studied factor to the probability (*P* value). Mixed ANOVA divides factors into random and fixed factors. The effect of a random factor is estimated as the percentage of embryo variance that can be related to this factor. Besides the residual factors, the oocyte and sperm factor were analyzed as random factors. In contrast, sperm motility, morphology and concentration were analyzed as fixed factors and were characterized by the corresponding regression coefficients showing, e.g., how the embryo features depend on the sperm characteristics.

FIGURE 1

Pearson's correlation between the average embryo grade of two recipients of the same oocyte donor. Recipient couples of the same OD procedure were randomly divided between the two axes.



Salumets. Effect of gametes on embryo quality. Fertil Steril 2002.

RESULTS

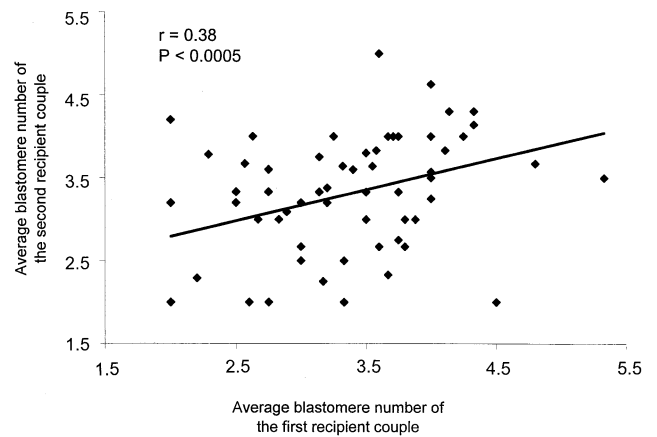
The mean age (\pm SD) of the oocyte donors was 29.4 ± 4.1 years (range, 21–36 years) and of the recipients, 33.8 ± 4.9 years (range, 23–49 years). A total of 1,070 oocytes were aspirated in 59 oocyte collections with an average of 18.1 oocytes per collection. The oocytes were inseminated with 1.3×10^5 , 1.3×10^5 , and 1.1×10^5 progressively motile spermatozoa when patients possessed <4% ($n=13$); 4–14% ($n=69$), and >14% ($n=36$) morphologically normal sperm cells, respectively.

The overall fertilization rate was 60.4% (646/1,070). The fertilization rate for patients with <4% morphologically normal sperm cells was 52.5% (63/120). Patients with 4%–14% normal cells had a fertilization rate of 62.7% (379/604), and patients with >14% normal cells had a fertilization rate of 59% (204/346). Only a marginal difference ($P=.05$) in the fertilization rates between the first and the second group of patients was observed, while no significant difference was seen between the first and third group of patients.

The investigation of the different factors involved in embryo development included statistical analysis of 646 embryos, i.e., 5.5 embryos per recipient couple. Pearson's correlation analysis revealed a strong correlation ($r = 0.67$, $P < .0001$) between the embryo morphology of the first recipient couple and that of the second recipient couple of the same OD procedure (Fig. 1). A weaker correlation ($r = 0.38$, $P < .0005$) was found between the average blastomere number of the first recipient couple and that of the second

FIGURE 2

Pearson's correlation between the average blastomere number of two recipients of the same oocyte donor. Recipient couples of the same OD procedure were randomly divided between the two axes.



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recipient couple (Fig. 2). These findings were subsequently corroborated by mixed ANOVA showing that the oocyte had a considerable effect ($P < .0001$) on embryo morphology and a weaker effect ($P=.01$) on the blastomere cleavage rate (Table 1).

Further analysis with mixed ANOVA demonstrated a significant ($P=.015$) sperm effect on the blastomere cleavage rate but not on embryo morphology (Table 1). More specifically, the sperm morphology as determined according to strict criteria was shown to be positively associated

TABLE 1

The results of mixed ANOVA showing the influence of oocytes and spermatozoa on embryo morphology and the blastomere cleavage rate. Oocyte and sperm factors were analyzed as random factors, whereas the sperm characteristics were estimated as fixed factors.

	Embryo morphology	Blastomere cleavage rate
Oocyte factor	$P < .0001$	$P = .01$
Sperm factor	NS	$P = .015$
Sperm count	NS	NS
Sperm morphology	NS	$P = .03$
Progressive motility before semen preparation	NS	NS
Progressive motility after semen preparation	NS	NS

Note: NS = not significant.

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($P=.03$) with the blastomere cleavage rate. However, other sperm characteristics such as sperm concentration and progressive motility before and after sperm preparation did not influence the blastomere cleavage rate. Furthermore, there was no correlation between sperm characteristics and embryo morphology.

In the IVF cycles of 31 recipients, elective one-embryo transfer was carried out, and in 75 cycles, two embryos were transferred. Twelve patients had all the embryos cryopreserved for future use. The overall delivery rate after 106 ETs was 30.2% (32/106). The delivery rate after elective one-embryo transfer was slightly higher (10/31, 32.3%) than that after transfer of two embryos (22/75, 29.3%). The only factor that slightly and negatively impacted ($P=.045$) the delivery rate was the number of embryos transferred, i.e., it was somewhat higher after one-embryo transfers than after two-embryo transfers.

DISCUSSION

In comparison with previous studies demonstrating the influence of oocytes and spermatozoa on early embryonic development, the value of this analysis lies in its unique study design. Young and fertile oocyte donors provided high-quality eggs, which were randomly divided between two recipient couples and inseminated by sperm from two men. This study design enabled us to determine the respective influences of the oocyte and the spermatozoa on human preimplantation embryo development.

Our results are in accordance with previous studies that provide evidence for an association between the oocyte and embryo quality (4–7). Pearson's correlation analysis disclosed a significant correlation between the embryo morphology of the first recipient couple and that of the second recipient couple so that both couples of the same oocyte donor had embryos of similar quality. As both recipients obtained the oocytes from the same donor, it was the effect of the oocyte rather than the spermatozoa that influenced the embryo quality. These results were subsequently corroborated by the analyses using the mixed ANOVA procedure in which the impact of the oocyte on embryo development was estimated by comparing the variability of embryo quality between different OD cycles. It was found that the oocyte has a profound effect on embryo morphology and less of an effect on the blastomere cleavage rate.

The significance of oocyte quality on early embryonic development may be due to an intensive accumulation of cytoplasmic organelles, proteins, and RNA in the cytoplasm of the oocyte during the final stages of oocyte development (23). This endowment of organelles and molecules is essential for normal embryo development in the first 2 days during which the embryonic genome is silent (24). It has been shown that multiple factors are important in influencing the oocyte quality. These include the etiology of infertility (25–

27), the age of the patients (28–31), and the type of ovarian stimulation (32–36).

There is an ongoing debate regarding the influence of sperm factors on early embryonic development (8). In a recent study exploiting a donor oocyte sharing program, it was shown that the sperm effect on embryo development may already be detectable as early as the zygote stage (13). In that study, the zygote-stage morphology was compared between two recipient couples and a clear difference in the proportion of zygotes with abnormal pronuclear morphology was found (13). According to the investigators, the zygotes with abnormal morphology tended to cleave more slowly and exhibited comprehensive fragmentation and blastomere irregularities (13, 37).

Other studies have, however, demonstrated a later onset of sperm effect on embryonic development. Impaired sperm morphology was shown to result in reduced blastomere number (10) and poor embryo quality (9) 2 days after insemination. Significantly more information about the putative effect of sperm cells on embryo quality has been obtained from the extended culture of human embryos to the blastocyst stage. It has been ascertained that the blastocyst formation rate as well as the blastocyst morphology were significantly lower when sperm with impaired quality were used in fertilization of oocytes in conventional IVF procedures (14–16). Contrasting with the results of these studies, some reports have indicated lower fertilization rates but normal embryo development after IVF in patients with poor semen quality (11, 12).

In the current study, a significant sperm effect on the blastomere cleavage rate was found by comparing the variability of the embryo quality between two recipient couples of the same oocyte donor. In addition, a positive association between sperm morphology and the blastomere cleavage rate was demonstrated. In other words, faster blastomere cleavage rate may be expected in patients with a higher proportion of normal sperm cells than in patients with impaired sperm quality.

The finding that sperm morphology may be an important prognostic factor for the blastomere cleavage rate is particularly interesting in light of the numerous studies that show that the blastomere cleavage rate is the most important determinant of the developmental potential of the embryo (38). For example, the implantation rate of embryos having at least 8 cells before 55 hours post-fertilization has been shown to be better than the implantation rate of embryos having less than 8 cells (39). Furthermore, it has been suggested that the transfer of 4-cell embryos even in the presence of minor fragmentation should be preferred to the transfer of 2-cell embryos with no acellular fragments (40). Another study indicated a higher implantation potential for embryos that had reached the 2-cell stage 25 hours after insemination (41). The value of early cleavage in embryo selection has also been confirmed in intracytoplasmic sperm

injection (ICSI) (42). The fact that the time of fertilization is precisely controlled in ICSI indicates that the early cleavage of embryos is influenced by some intrinsic factors within the embryo (42). In the present study, we did not assess the relationship between sperm quality and early cleavage, and further studies will be necessary to clarify this issue.

There is little information available about the mechanism whereby the sperm cell could influence embryo development. It is generally assumed that only maternally produced transcripts and proteins govern the first two cell divisions in preimplantation human embryo development as genes are not expressed until about the 4- to 8-cell stage (24). Hence, it is plausible that sperm could affect the development only after the activation of the embryonic genome. However, the sperm cells are known to carry some epigenetic factors regulating embryo development. The most important cellular contribution of the sperm cell to the zygote is the centrosome. During embryo development, the sperm centrosome forms the poles of the mitotic spindle, thereby regulating the first and subsequent cell divisions. Indeed, the indispensability of the centrosome for normal embryo development has been substantiated by the study of Asch et al. (43), which demonstrated that centrosome defects might lead to disorders in fertilization and early embryonic development.

In addition, we cannot neglect the effect of sperm DNA packaging on early embryonic development. Dissimilarities observed in chromatin packaging between different sperm samples (44) might well result in defective DNA decondensation, pronuclear formation, and delayed cell division events. Several studies have pointed out the association between impaired sperm morphology and either increased DNA damage (45, 46) or poor chromatin packaging (46, 47), helping us to understand the observed correlation between poor sperm morphology and reduced blastomere cleavage rate.

All of the above-mentioned studies, including ours, that have ascertained the influence of spermatozoa on embryo development have been based on a routine IVF procedure in which the number of blastomeres observed 2 days after insemination may have been influenced by the timing of fertilization. The time of fertilization depends on the maturity of the oocytes: less mature oocytes are fertilized later than mature oocytes, leading to delayed fertilization and early cleavage (48). In this study, the oocyte maturity was checked according to nuclear maturation on the day after insemination and the proportion of immature oocytes was found to be similar in both recipient couples of the same OD procedure (data not shown). Hence, we could minimize the possible influence of oocyte maturity on the time of fertilization.

Another key factor that may lead to delayed fertilization is impaired sperm morphology (10). Fertilization is a complicated process that occurs in a stepwise fashion. It has been argued that sperm cells with pathological findings could

have difficulties in completing one of the steps involved in the egg-sperm interaction (10). Indeed, it has been demonstrated that the human zona pellucida is highly selective for spermatozoa with normal morphology (49). In contrast to the study by Ron-El et al. (1991) (10), almost all patients participating in the current study had normal sperm quality and no difference was observed in the fertilization rates between the so-called poor prognosis pattern group with <4% morphologically normal sperm cells and patients with >14% normal sperm cells.

It has also been demonstrated that prolonged oocyte exposure to a high concentration of spermatozoa may have an adverse effect on subsequent embryo development because of the reactive oxygen species produced by both normal and abnormal spermatozoa as well as by activated leucocytes (50–54). Retarded embryonic growth has been shown to be associated with the group of oocytes inseminated with a high concentration of spermatozoa (53). However, in our study, similar insemination concentrations were used. Thus we believe that the variability in cell numbers observed between sibling embryos of the same oocyte donor are caused by differences in blastomere cleavage rate, unrelated to the time of fertilization.

The factors related to the success of the whole IVF procedure were also estimated within our study group. This analysis was, however, more complicated because it involved not only the embryo characteristics, but also the number of embryos transferred, the uterine receptivity, and the women's ability to carry a pregnancy to term. When the delivery rates were compared between the two recipient couples, the only factor that was slightly related to the IVF outcome was the number of embryos transferred. Contrary to expectations, this relation was negative, i.e., the delivery rate was slightly higher after one-embryo transfers than after two-embryo transfers. This is likely to indicate that patients who underwent elective single ET had better embryos and were better fit to carry the pregnancy to full term.

The results of this study provide compelling evidence that embryo morphology 2 days after insemination is predominantly determined by the properties of the oocyte, whereas the blastomere cleavage rate is simultaneously influenced by both the sperm cell and the oocyte. Future research should be focused on understanding the exact mechanism whereby the oocyte and spermatozoa may influence early embryonic development.

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