

# Impact of sperm dna fragmentation on pregnancy



**ASSIGN  
BUSTER**

The influence of human sperm DNA fragmentation on the rate of fertilization and sperm nuclear condensation after in vitro maturation of oocyte in stimulated intracytoplasmic sperm injection cycles

## ABSTRACT

**Background:** The aim of this study was to evaluate the impact of sperm DNA fragmentation (SDF) on the fertilization rate and sperm nuclear decondensation after intracytoplasmic injection of sperm (ICSI) into cumulus-free germinal vesicle (GV) oocytes from stimulated cycles.

**Methods:** We analyzed 50 ICSI cycles containing at least two GV oocytes at retrieval day. The GV oocytes were cultured for 24 hours. Oocytes that liberated polar bodies were injected by processed semen which evaluated their SDF level. According to SDF, the data categorized into two groups; group I:  $SDF \leq 30\%$  and group II:  $SDF > 30\%$ . Fertilization (presence of two pronuclei), was checked 16-19 hours after ICSI. Unfertilized oocytes were stained by Hoechst 33258 and examined by fluorescence microscopy to evaluate the undecondensed sperm head in oocyte. The rates of maturation, fertilization in fertilized IVM oocytes and the percentage of undecondensed sperm in unfertilized oocytes were assessed according to SDF.

**Results:** From among 146 GV oocytes subjected to IVM, 101 (69 %) developed to metaphase II. The fertilization rate of IVM oocytes in group II ( $45.43 \pm 6.14$ ) was significantly lower than that in group I ( $63.88 \pm 6.27$ ) ( $P < 0.05$ ). Moreover, group I, had 25% of their unfertilized oocytes containing sperm that remained condensed, while group II, had a significantly higher number (53 %) of unfertilized oocytes containing condensed sperm.

Conclusions: Paternal chromatin damage had a negative effect on the rate of fertilization in GV oocytes matured in vitro and could lead to an increase in the percentage of undecondensed sperm in IVM oocytes from stimulated cycles.

Keywords: sperm DNA fragmentation, germinal vesicle oocyte, in vitro maturation, ICSI- fertilization, sperm nuclear condensation

## INTRODUCTION

In assisted reproductive technology (ART), controlled ovarian stimulation (COH) used to achieve multifollicular recruitment in order to increase in the number of collected oocytes on the puncture day. Yet, not all follicles respond to the stimulation of the external gonadotropin. In fact, from among all the retrieved oocytes, about 15-20% are immature [1, 2] and, sometimes a majority of or the whole collected oocytes are germinal vesicle (GV) oocytes [3] Some of these immature oocytes are capable of liberating their first polar body in laboratory conditions, and can be used as a valuable source of oocytes for sperm injection in ICSI [4].

Despite the several clinical studies on the outcome of intracytoplasmic sperm injection into ooplasm in vitro matured human oocyte derived from stimulated ovaries[5, 4, 6], controversy continues to exist on the fertilization rate and developmental competence of these immature oocytes.

In ICSI, the embryologist may randomly select the spermatozoa often from a population with a relatively high incidence of fragmented DNA [7]. That is why a possible risk in ICSEA is using damaged DNA sperm cell, even if their

appearance and motility look normal [8], which can endanger the fate of fertilized ICSI eggs.

In spite of the fact that numerous studies have evaluated the impact of SDF on the ICSI-fertilization rate with in vivo matured MII oocytes, a literature search revealed that no study has noted the role of sperm chromatin, that serves as donator of half of embryo genome, in the rate of fertilization in vitro matured oocytes derived from stimulated cycles .

The number of mature oocytes (MII), however, is not sufficient in some of the infertile couples and/or only immature oocytes are available in puncture day from stimulated cycles. Moreover, in processed semen, the level of sperm DNA fragmentation is relatively high that cannot be recognized via conventional semen analysis. In such cases, we routinely use intracytoplasmic injection of morphologically normal sperm with unknown DNA fragmentation level into in vitro matured oocytes.

Considering the fact that the fertilization rate after sperm injection to these immature oocytes varied from 44. 2%-79. 8% [5, 4, 6], a substantial percentage (20-55%) of the oocytes are unfertilized and thus potentially wasted. Having in mind the facts that, as a predicative test for fertilization capability of sperm and oocyte after ICSI, male chromatin deconstruction could implemented and also injection of sperm containing damaged DNA into in vivo matured oocytes may play a role in failing the decondensation process and thus result in failure of pronuclei formation and fertilization, in the current study, we cultured GV oocytes for 24 hours and evaluated the rates of fertilization according to SDF level. Moreover, we examined the fate

of sperm in unfertilized oocytes to evaluate the percentage of undecondensed sperm in these oocytes with respect to the SDF level in processed semen.

## RESULTS

A total of 146 immature oocytes from 50 stimulated cycles were collected from women with average ages of  $31.2 \pm 1.6$  and  $30.5 \pm 1.3$  in group I and II, respectively. From among the GV oocytes subjected to IVM, 69% progressed to MII stage 24 hours after culturing (Table 1).

The total number of injected oocytes after in vitro maturation as well as fertilization rate is given in Table 2. The number of injected oocytes was 68 and 50 in group I and group II, respectively. The rate of fertilization in group I was significantly higher than that in group II ( $P < 0.05$ ).

As shown in Table 2, a total of 55 unfertilized oocytes were stained via fluorochrome Hoechst 33258. About 3.5% of the stained oocytes did not demonstrate presence of any sperm inside, and sperm ejected, 3.7% and 3.6% in group I and II, respectively. In the remainder of the oocytes examined in group I, ~25% contained sperm that remained condensed, whereas 60% of the sperm were either decondensing or decondensed. In group II, ~53% of the unfertilized oocytes were found to have undecondensed sperm head and ~38% contained decondensed or decondensing sperm.

The rate of the sperm head that remained condensed in group I was significantly lower when compared with that in group II ( $P < 0.05$ ). Also, no significant difference was found between group I and II in the percentage of

decondensing or decondensed sperm ( $P > 0.05$ ). Moreover, 15% and 9% of unfertilized oocytes were categorized as “others”; in group I, four unfertilized oocytes were not interpretable and in group II, two.

## DISCUSSIONS

The present study demonstrated that paternal chromatin damage had a negative effect on the rate of fertilization in cumulus-free germinal vesicle oocytes matured in vitro from stimulated cycles. Also, we found that in the unfertilized IVM oocytes derived from intracytoplasmic injection of sperm with high level of SDF in processed semen, the percentage of the remaining condensed sperm is higher than that of  $SDF \leq 30\%$ .

The rate of in vitro maturation of oocytes in stimulated cycles, as reported in the literature, varies from 35% to 75% [11-13, 3, 14]. The variation in the reported maturation rates in these studies are probably the result of implementing different study conditions including: duration of the in vitro culture, media components and addition of different supplements; hormones, vitamins, antioxidants, growth factors and serum [15, 12, 16]. Escrich et al. [13], reporting the highest level of maturation rate (74.8%) in the stimulated cycle to date, cultured cumulus-free GV oocytes in human Tubal Fluid medium (hTF) for 48 hours and demonstrated that most of the denuded GV oocytes collected from stimulated ovaries extrude the first polar body (PB) spontaneously after a minimum of 22 hours.

In the present study, 69% of cumulus-free GV oocytes, collected following superovulation, progressed to MII after 24 hours in vitro culture; a finding that is close to the results achieved by Farsi et al. [14] who demonstrated

<https://assignbuster.com/impact-of-sperm-dna-fragmentation-on-pregnancy/>

that 70% of the retrieved GV oocytes matured to MII in GI media after 24-30 hours of incubation.

In stimulated ICSI cycles, denudation of cumulus cells is inevitably performed following oocyte retrieval in order to evaluate the oocytes' nuclear maturity status, thus we, also, used cumulus-free germinal vesicle oocytes for in vitro culture. However, Kim and coworkers [11] investigated the impact of the presence of cumulus cells on the maturation rate of GV oocytes in stimulated cycles and reported that the presence or absence of cumulus cells did not have any significant effect on the maturation rate of GV oocytes from follicles primed with gonadotropin [11].

Yet, immature human oocytes that are retrieved from stimulated cycles have fertilization capability and can produce early embryos in vitro [17], although the previous studies reported on different fertilization rates, varied from 44.2% to 79.8% [5, 4, 6]. One possible explanation why the rates of fertilization in ICSI-IVM oocytes vary in literature may be that these studies ignore to note the significance of the sperm contribution to fertilization. The paternal genome quality is undoubtedly the important factor determining how successfully fertilization process is carried out and so it has to be considered in intracytoplasmic injection of sperm, because in spite of their normal appearance, some sperm that are selected for injection contain fragmented DNA [8] and are unrecognizable in routine sperm analysis.

So far, many studies have evaluated the impact of SDF on the rate of ICSI-fertilization from oocytes that already mature at the moment of oocytes denudation [18, 19], however the results have been contradictory. Some

clinical studies have found no significant correlation between sperm DNA damage and fertilization rates in vitro [20-23, 18], whereas others reported on a negative correlation between sperm DNA damage and fertilization rates [7, 24-27].

In the current study however, for the first time, we reported the negative effect of SDF on the fertilization rate in cumulus-free immature human oocytes derived from stimulated and ICSI cycles, so that the fertilization rate was significantly lower in group II as opposed to that in group I. The fertilization rates in patients with SDF > 30% (group I) (~64% ) from GV oocytes matured in vitro from stimulated ovaries were close to those obtained in the study reported by Farsi et al. (65%) [14] and Reichman et al. (62. 1%) [3]. Similar to our study, in both these studies, stripped GV oocytes were cultured for an overnight and sufficient motile sperm of gross normal morphology were implemented for ICSI. Sometimes, despite successful injection of sperm into each oocyte, the rates of fertilization happen to be poor. In group II with SDF > 30%, our fertilization rate was close to the study reported by Shu et al. (44. 2%) [4], indicating that a significant proportion (56%) of the oocytes are unfertilized and thus potentially wasted.

Unlike our study, none of these studies specified the sperm DNA fragmentation level in ICSI cycles, while Sakkas et al. [28] demonstrated that intracytoplasmic injection of sperm with a high level of abnormalities in the sperm chromatin into in vivo matured oocytes may lead to decondensation failure in sperm nucleus and thus an apparent failure of fertilization.

However, what was a gap in our current knowledge was understanding the role of sperm DNA fragmentation in the sperm nucleus decondensation and

<https://assignbuster.com/impact-of-sperm-dna-fragmentation-on-pregnancy/>



fertilization in human unfertilized oocytes that matured in vitro. Accordingly, in the present study, in order to examine the sperm fate in IVM oocytes based on SDF level, we stained unfertilized IVM oocytes via the flurochrome dye. From among the oocytes subjected to staining, approximately 3.5% displayed no sign of sperm inside the ooplasm. Flaherty et al. [29], Sakkas et al. [28], and Lopes et al. [30] reported that 19%, 16%, and 16.7% of the oocytes examined, in that order, had ejected the sperm after ICSI. They suggested that the ejection of the sperm is most probably because of a technical error during the ICSI procedure, although contrary to their reports, in the present study, ejection of the sperm from the oocyte into perivitelline space (PVS) only occurred in ~3.5% of all of the stained unfertilized oocytes.

However, data obtained in the current study revealed that patients with SDF > 30% in processed semen had a significantly higher number of unfertilized oocytes (53%) that contained condensed sperm. Our data is in agreement with that in Sakkas et al. [28] who examined the presence of DNA damage in the sperm of patients treated with ICSI making use of in-situ nick translation and evaluated unfertilized mature MII oocytes obtained from the stimulated ovaries via the flurochrome Hoechst 33342 to determine whether a relationship exists between failure of fertilization and sperm chromatin damage. Separating patients into two groups based on their sperm chromatin abnormality (Chromomycin A3 (CMA3) > 30% and endogenous nicks of > 10%), they showed that there is no difference between the fertilization rates after ICSI. They also found that patients with a high degree of chromatin damage (nicks > 10% of their sperm) had a significantly higher

number of unfertilized oocytes (~50%) that contained condensed sperm. As conclusion, they claimed that a significant factor resulting in a failure of sperm nucleus decondensation and fertilization could be paternal chromatin.

Also, Lopes and colleagues [30] investigated the percentage of maternal and/or paternal fragmented DNA in unfertilized oocytes after ICSI using a modified version of the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin end-labeling (TUNEL) method. They found that 50% of oocytes that displayed no pronuclear formation, contained chromatin with damaged DNA and hypothesized that intracytoplasmic injection of sperm containing abnormal chromatin into oocyte will probably result in failure of sperm decondensation and fertilization.

Moreover, Flaherty et al. [29] showed that ~30% and 10% of the unfertilized activated and MII oocytes, respectively, contained undecondensed sperm head, irrespective of sperm DNA integrity. Nevertheless, some studies found no correlation between chromatin decondensation test and fertilization rate [31-33].

In agreement with Sakkas et al.[28], our data showed that high values of SDF > 30% lead to unfertilized oocytes that contain higher percentages of condense-remaining sperm. Consequently, a high level of SDF in processed semen for ICSI may hamper the completion or initiation of decondensation and therefore leading to a failure of fertilization regardless of the oocyte possessing the necessary mechanism to initiate decondensation.

Similar to Sakkas et al [28] study, in the current study, we investigated the fate of the sperm but in IVM oocytes and not in in vivo matured oocytes, and <https://assignbuster.com/impact-of-sperm-dna-fragmentation-on-pregnancy/>

we cannot claim, either, that the fertilization failure is totally as a result of a sperm defect; it also seems possible that damaged DNA sperm may have a role in a failure in the decondensation process, nevertheless, this is the first study demonstrating that damaged DNA may contribute to failure of sperm decondensation after intocoplasmic sperm injection into in vitro matured cumulus-free germinal vesicle oocytes.

In conclusion, in the present study, we found that SDF > 30% can results in a decrease in the fertilization rate and an increase in the percentage of undecondensed sperm head in denuded germinal vesicle. Therefore, we suggest that analyzing the DNA integrity of processed sperm can enhance the ability to predict the rate of fertilization after in vitro maturation oocytes, especially when oocytes have failed to mature adequately in stimulated cycles and that we have to use in vitro matured oocytes in order to increase the number of embryos available for transfer or cryopreservation.