

# **Correlations of DNA Fragmentation with Apoptosis and Motility in Frozen-thawed Human Spermatozoa**

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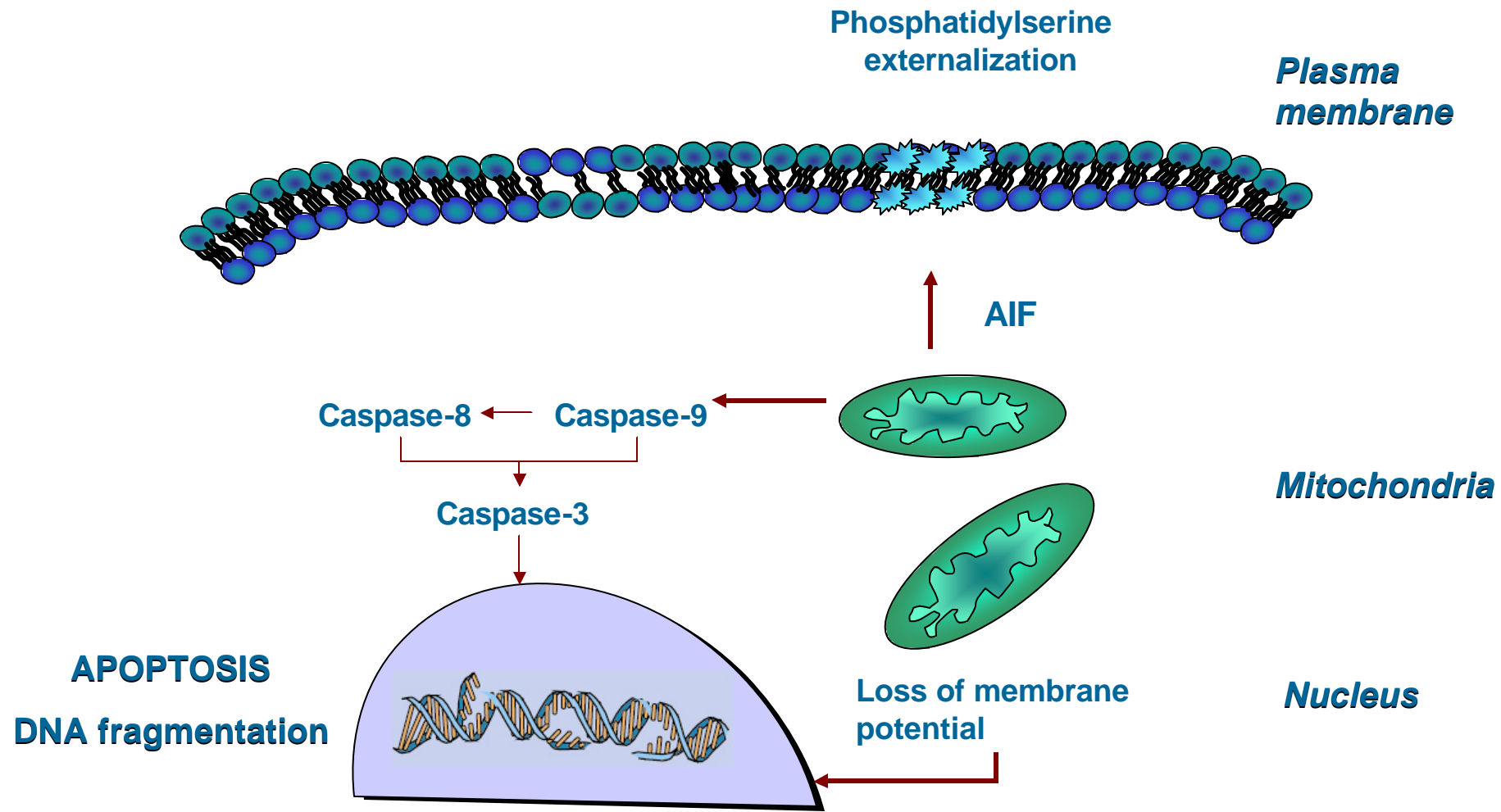
# Apoptosis in Male Germ Cells

- Controls the overproduction of male germ cells
- Apoptosis (or apoptosis-like manifestations) are found in ejaculated human spermatozoa
- Although not well defined, deregulated apoptosis is associated with male infertility

(Said et al, Hum Reprod Update, 2005)



# Apoptosis in Human Spermatozoa



# Apoptosis (Late)

- **Sperm DNA damage:**
  - One of the most commonly studied, controversial causes for male infertility that may pass undetected due to lack of its proper assessment
  - In addition to apoptosis, may be due to oxidative stress and defective chromatin packaging



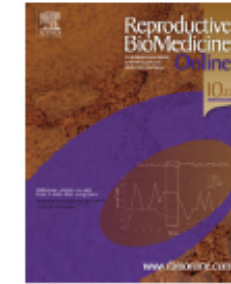
# Sperm Cryopreservation

- Manifestations of cryo-injury may include sperm DNA fragmentation
- Despite recent methodological advances, protocols for cryopreservation-thawing exert detrimental effects on spermatozoa






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REVIEW

## Implication of apoptosis in sperm cryoinjury

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**Abstract** Apoptosis is an ongoing physiological phenomenon that has been documented to play a role in male infertility, if deregulated. Caspase activation, externalization of phosphatidylserine, alteration of mitochondrial membrane potential and DNA fragmentation are markers of apoptosis found in ejaculated human spermatozoa. These markers appear in excess in subfertile men and functionally incompetent spermatozoa. Sperm cryopreservation is a widely used procedure in the context of assisted reproductive techniques. Cryopreservation and thawing is a procedure that inflicts irreversible injury on human spermatozoa. The damage is manifested by a decrease in recovery of viable spermatozoa with optimum fertilization potential. This review describes the implication of apoptosis as one of the possible mechanisms involved in sperm cryoinjury. Evidence shows significant increase in some apoptosis markers following cryopreservation and thawing. On the other hand, the increase in sperm DNA fragmentation following cryopreservation and thawing requires further investigation. Specific technical measures should be applied to minimize the induction of apoptosis in human spermatozoa during cryopreservation and thawing. These include standardization of freezing protocols and cryoprotectant use. Selection of non-apoptotic spermatozoa may also prove to be of benefit. 



# Objective

- To identify the relationships between DNA fragmentation, apoptosis and motility in frozen-thawed human spermatozoa



# Materials and Methods

Proven Fertile Donors (n=26), Semen Samples (n=61)

Cryopreservation (SMMG, 7% glycerol)

Thawing

*Evaluation*  
**Motility (%)**

**Flow Cytometric  
Assays**

**DNA Fragmentation**

*Evaluation*  
DNA Damaged (%)

**Annexin V**

*Evaluation*  
Non-apoptotic (%)



# Results

Post-thaw Data (n=61)

**Motility**  
(%)

**32.8 ± 9.3**  
**(8.0 – 51.0)**

**DNA Fragmentation**  
(%)

**15.3 ± 8.3**  
**(4.5 - 38.8)**

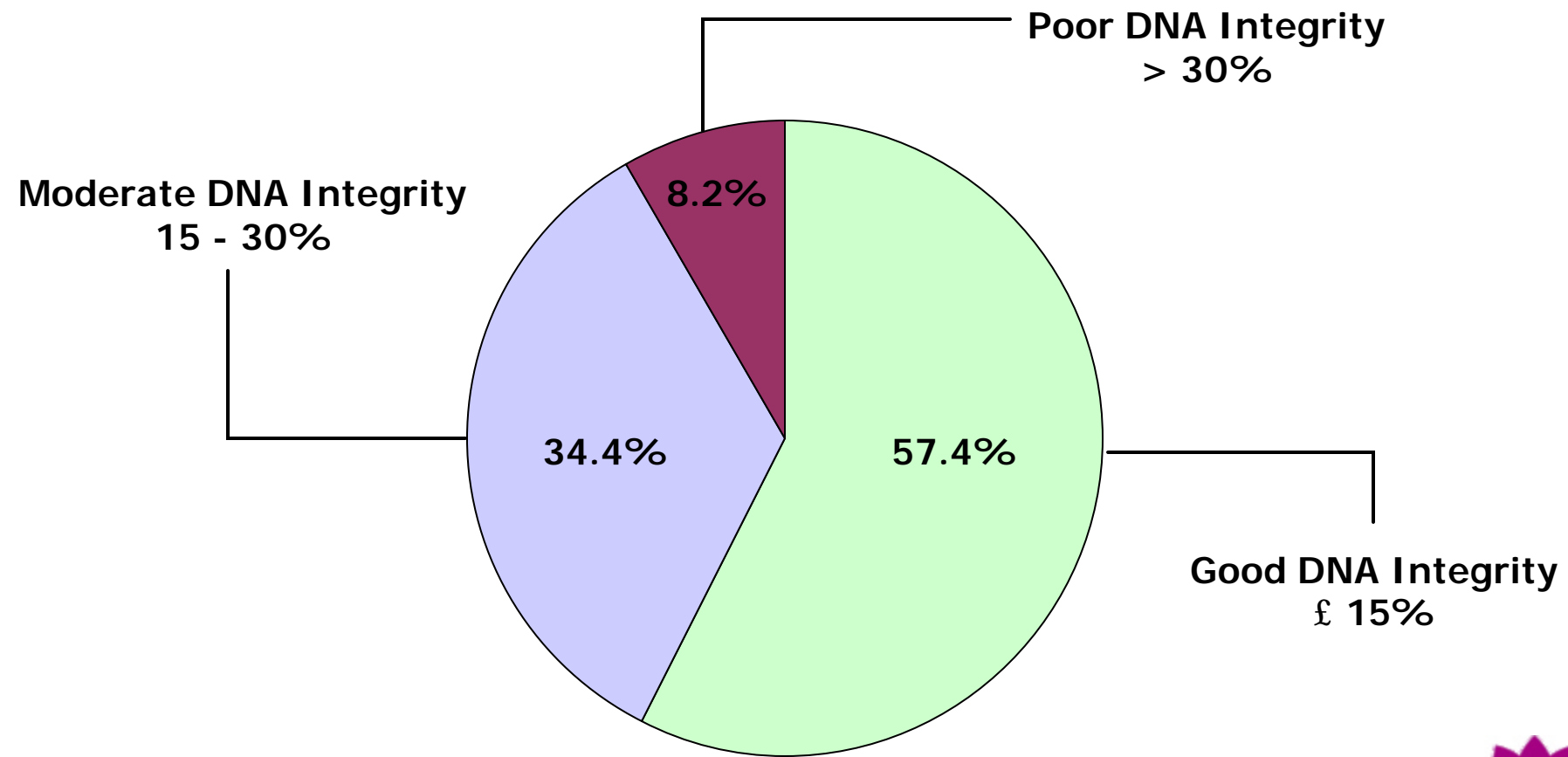
**Apoptosis**  
(%)

**31.3 ± 9.6**  
**(14.0 – 58.6)**



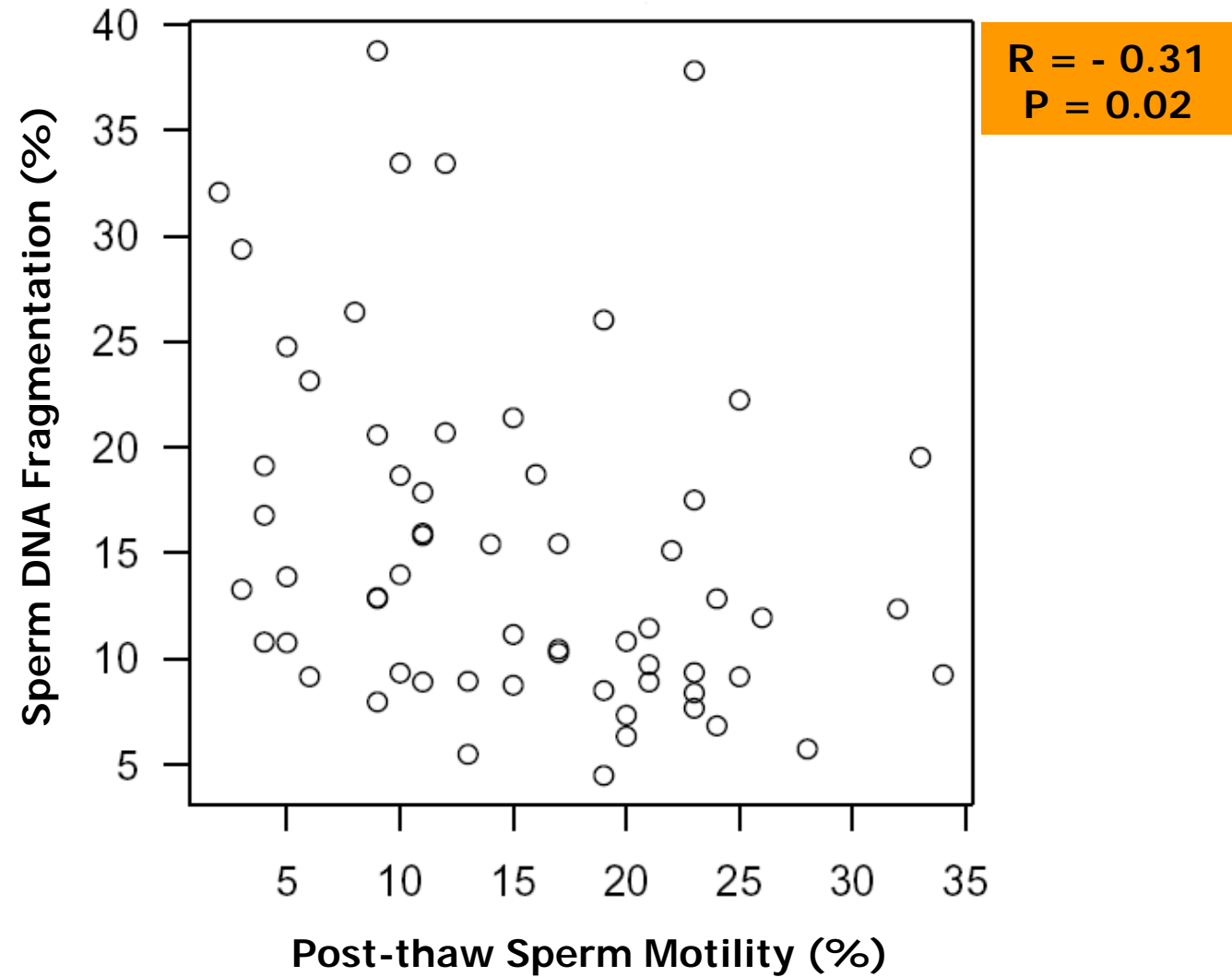
# Results

## Distribution of DNA Integrity within analyzed samples (n=61)



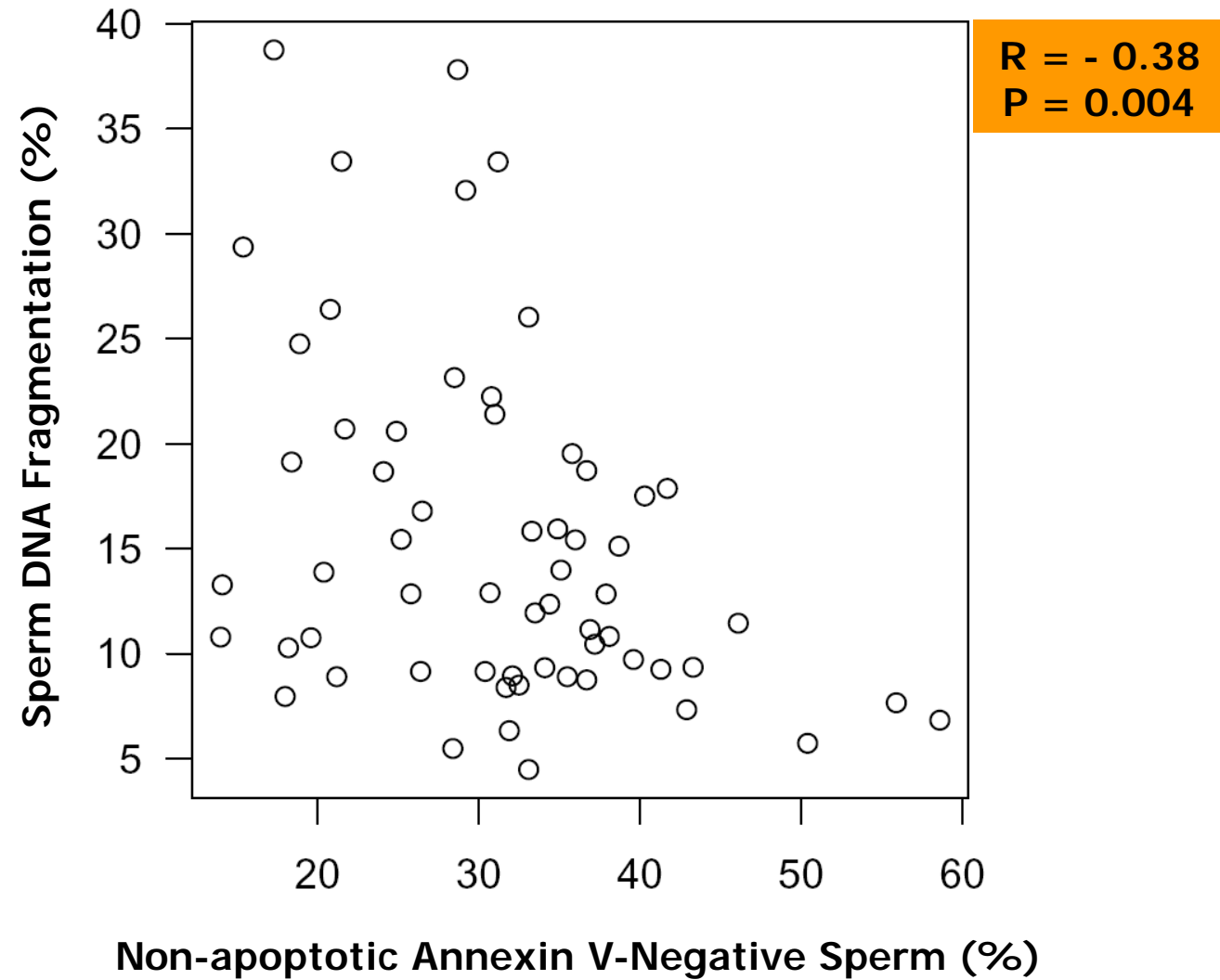
# Results

## Correlation: Sperm DNA fragmentation and Motility



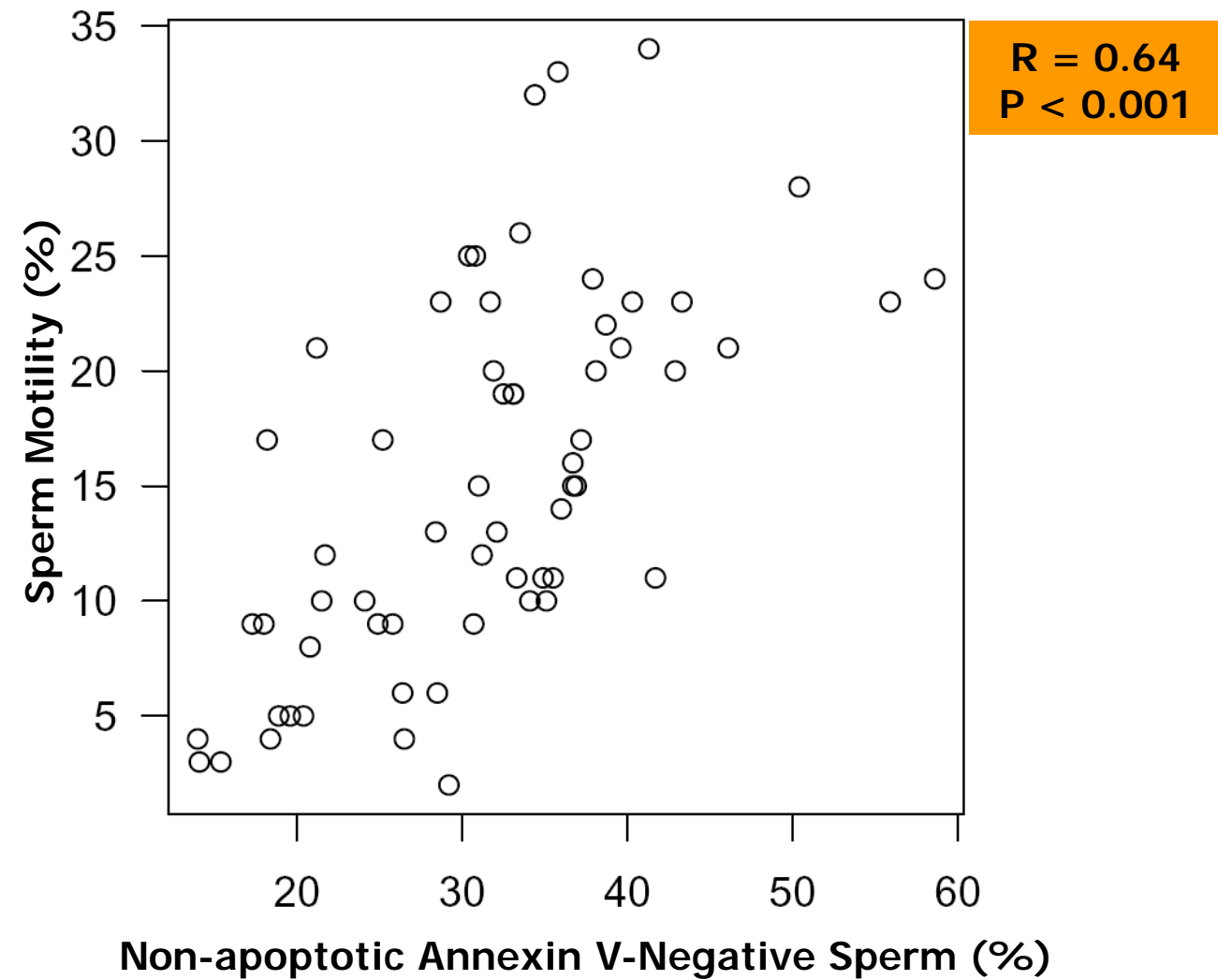
# Results

## Correlation: Sperm DNA fragmentation and Apoptosis



# Results

## Correlation: Sperm Motility and Apoptosis



# Conclusions

- **Spermatozoa with fragmented DNA do not appear in excess following cryopreservation-thawing (in proven fertile donors)**
- **Lower sperm DNA fragmentation and apoptosis are associated with higher motility post-thaw**



# Conclusions

- **DNA fragmentation is correlated with apoptosis in cryopreserved-thawed sperm**
- **Apoptosis may be one of the pathways leading to sperm cryoinjury and DNA fragmentation**



# Future Directions

- **A sperm selection method that aims at isolating non-apoptotic, motile spermatozoa should be developed and considered regarding its ability to increase DNA intact sperm following cryopreservation-thawing**



***Thank You***

