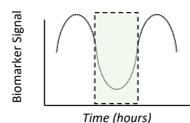


## INTRODUCTION

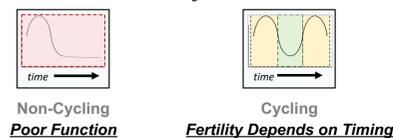
Freshly ejaculated mammalian sperm have poor fertilizing ability. Fertility is gained only after sperm undergo capacitation and the acrosome reaction [1, 2]. We have discovered that these maturation processes occur in sequential waves and among synchronized cohorts of sperm and not randomly among individual sperm leading to egg fertilization by vanguard sperm [3]. This has been termed the “sperm fertility cycle.”

### Discovery: The 4 Factors of The Male Fertility Cycle in Ejaculates

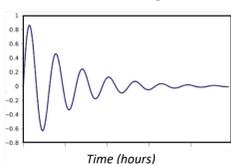
#### 1. Periodic Fertility Windows Called “Cycling”



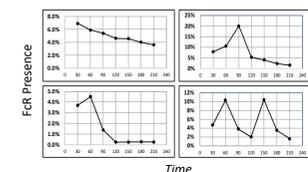
#### 2. Cycling & Timing Determines Fertility State



#### 3. Cycling Fades with Time -- and Fertility With it



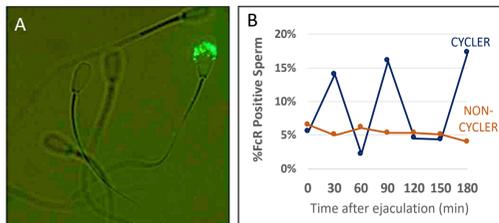
#### 4. Each Ejaculate is Different



## OBJECTIVE

We sought to understand if sperm fertility cycles impact advanced ART procedures such as IVF and ICSI and if this could be measured using a functional assay for sperm. The Arex Assay visualizes sperm maturation waves by identifying an Fc receptor that becomes detectable as sperm undergo functional changes.

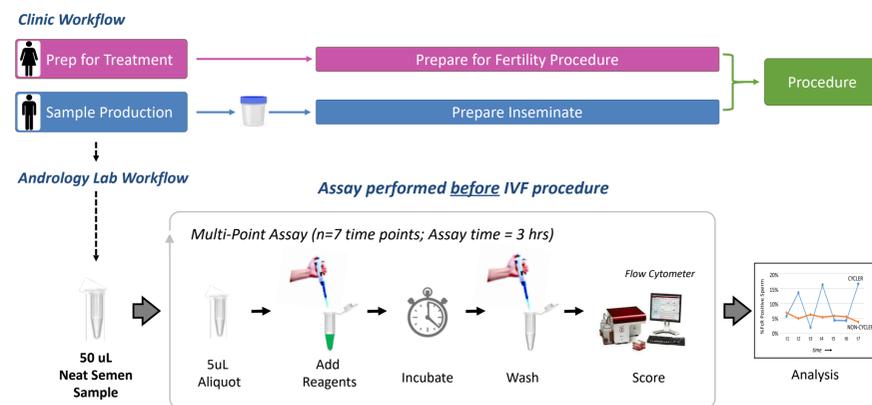
The Arex Assay measures the proportion of sperm that express FcR. (A) Sperm stain positive (green) for FcR. (B) The %FcR-positive sperm over time after ejaculation is plotted to visualize sperm cycling activity. Ejaculates are classified as either “cycling” or “non-cycling.” The highest fertility occurs at the minimum point of %FcR positivity. Non-cycling samples have low fertilization potential.



## METHODS & CYCLE OUTCOMES STUDIED

The Arex Assay measures functional Fc receptor levels (FcR). It was performed on inseminating semen samples of infertile couples undergoing IVF at a North American fertility center. Briefly, 5uL aliquots of semen were analyzed at 30-min intervals to determine the proportion of sperm that were expressing FcR and the temporal changes in FcR expression levels were analyzed. Semen samples were categorized as either “cycling” or “non-cycling,” depending on FcR expression patterns. Knowledge of Arex Assay findings was not used to alter the timing of IVF procedures. IVF success was defined as development of embryos to a stage suitable for freezing and transfer. Failure was defined as a procedure resulting in no frozen or transferable embryos. Notably, ICSI was not used. Also, semen assay timing was not consistent with procedure timing, which caused the assay to be less sensitive.

### Arex Assay Experimental Workflow Specific to IVF Study



### Cycle Outcomes of IVF Samples in the Study

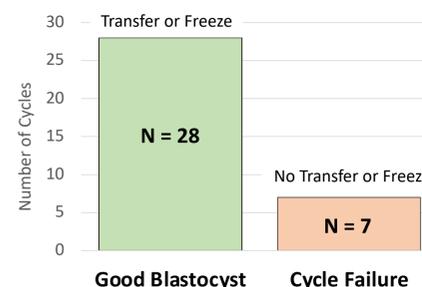
#### Study to assess the efficacy of Arex Assay in IVF procedures

- US Study Site
- Randomized
- Prospective

**Fresh Partner Sperm**

- N = 35 couples
- N = 35 IVF cycles

All samples were within nominal WHO semen reference ranges.

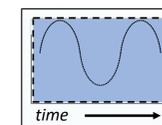


## RESULTS

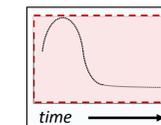
Among n=35 IVF cycles performed in 35 couples, 28/35 (80%) were successful while 7/35 (20%) had failed IVF, according to our criteria. Among successful IVF cases, 23/28 (82%) were associated with cycling sperm. Importantly, among IVF failures, 6/7 (86%) were associated with non-cycling sperm. The odds ratio for reproductive outcome among cycling vs non-cycling semen specimens was 27.6 (95% CI: 2.25, 1321.8).  $p=0.0017$ ; Fisher’s exact test).

### Arex Assay is Predictive of Cycle Outcome in IVF (Note: Sperm enters egg prior to cycle failure)

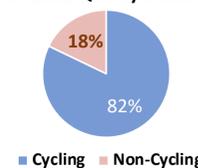
#### Cycling: **Functional**



#### Non-Cycling: **Poor Function**

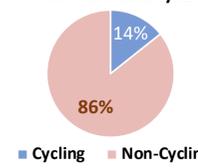


#### N = 28 IVF Cycles with Good Quality Blastocysts



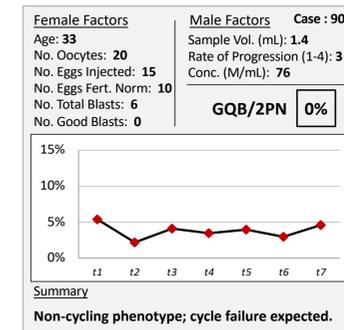
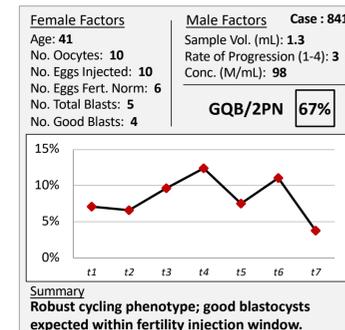
#### For N = 35 IVF Cycles

#### N = 7 IVF Cycles with No Blastocysts



### IVF Results Parallel ICSI Outcomes\*

Case reports illustrate the assay’s ability to identify phenotypic fertility function in clinical semen samples that impact ART outcome. The presence of “normal” semen parameters and adequate female-side retrieval values are not sufficient for good blast formation when sperm function is deficient per Arex Assay.



\* Presented with permission from Yazdekhashti, et al., PCRS 2023 [4], presented by Dr. Rebecca Holmes, CCRM Fertility, Boston MA.

## CONCLUSIONS

In this pilot study, IVF procedures performed with “cycling” semen specimens resulted in statistically significantly higher success rates than those performed with non-cycling specimens. Because cycling status was evaluated 2 hours prior to mixing of gametes, a cycling sample that faded over time and subsequently failed at IVF, would escape detection and confound interpretation of the cycling results. Such an ejaculate would appear to be functional but would have failed to cycle by procedure time. Future studies will be conducted with a peri-IVF design to eliminate this issue. The IVF data presented here are consistent with recent ICSI results obtained by Yazdekhashti, et al. [4]. Even when sperm from a non-cycling ejaculate are directly injected into the egg, good blastocysts are not formed. The Arex Assay has the potential to characterize the quality of sperm maturation processes and can be used to classify semen samples as either “fertile” or “infertile” based on IVF (without ICSI) outcomes.

### Summary

1. Ejaculated sperm mature in sequential waves to gain fertility.
2. Presence and timing of sperm cycles determines fertility.
3. Every ejaculate is different.
4. Sperm cycling dissipates with time after ejaculation.
5. Good outcomes are not observed in IVF with non-cycling, i.e., non-functional sperm, similar to results found in IUI and ICSI.

## REFERENCES

1. Austin (1951) Aust J Sci Res B 4: 581-596
2. Chang (1951) Nature 168: 697-698
3. Librach, et al., American Society of Andrology May 7-10, 2022; La Jolla, CA.
4. Yazdekhashti, et al., Pacific Coast Reproductive Society, March 22-26, 2023; Indian Wells, CA.

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