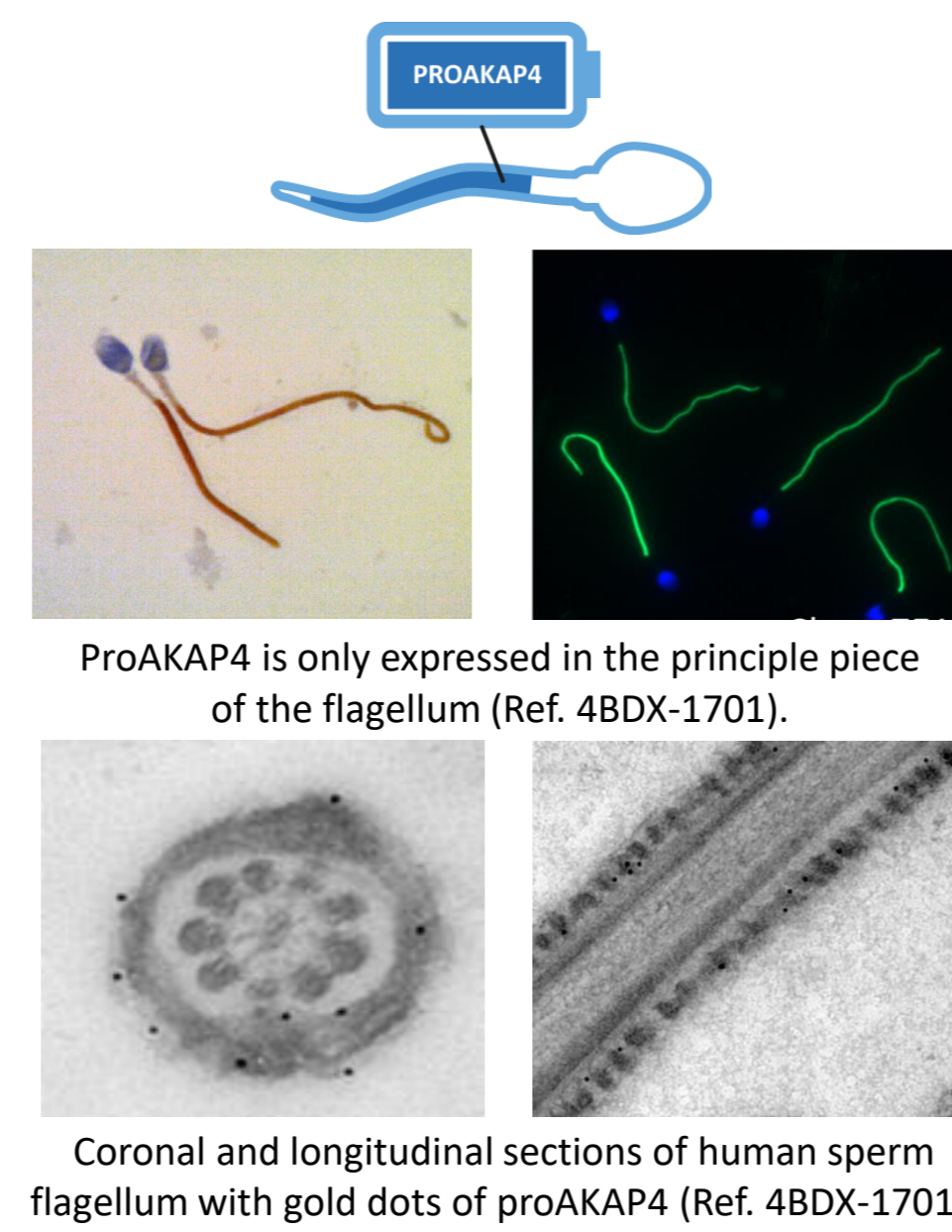


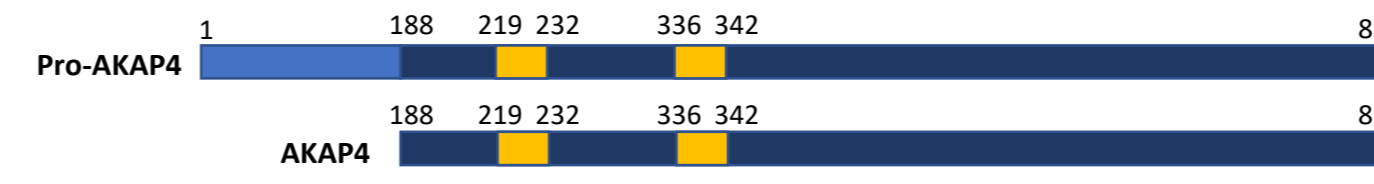
## Introduction

### The sperm protein proAKAP4 as a marker of sperm quality and fertility

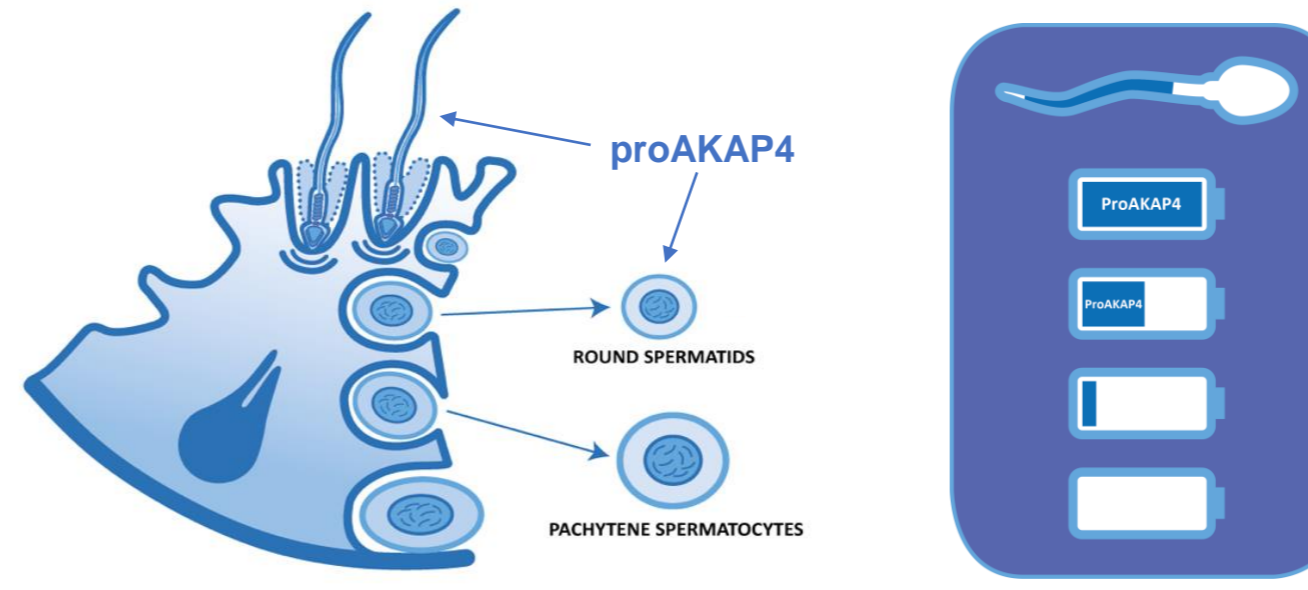
- ProAKAP4 is the protein precursor of the AKAP4 (A-Kinase Anchor Protein 4), a sperm specific protein
- Required for structure, motility, capacitation and fertilization
- Binds to the regulatory subunits of protein kinase A for activation (yellow boxes)
- Strictly localized in the fibrous sheath of the principle piece of the sperm flagellum
- Highly conserved between mammals (more than 70 % of homology)
- ProAKAP4/AKAP4 KO mice show a disorganized flagellum, immotile and infertile sperm (Miki et al. 2002)
- ProAKAP4 is described as a good indicator of sperm quality and fertility in bull, horse and pig (Sergeant et al. 2019).
- ProAKAP4 concentrations correlated with progressive motility in stallion (Blommaert et al. 2019) and in human spermatozoa (Jumeau et al. 2018).



### ProAKAP4/AKAP4 : A-kinase Anchor Protein



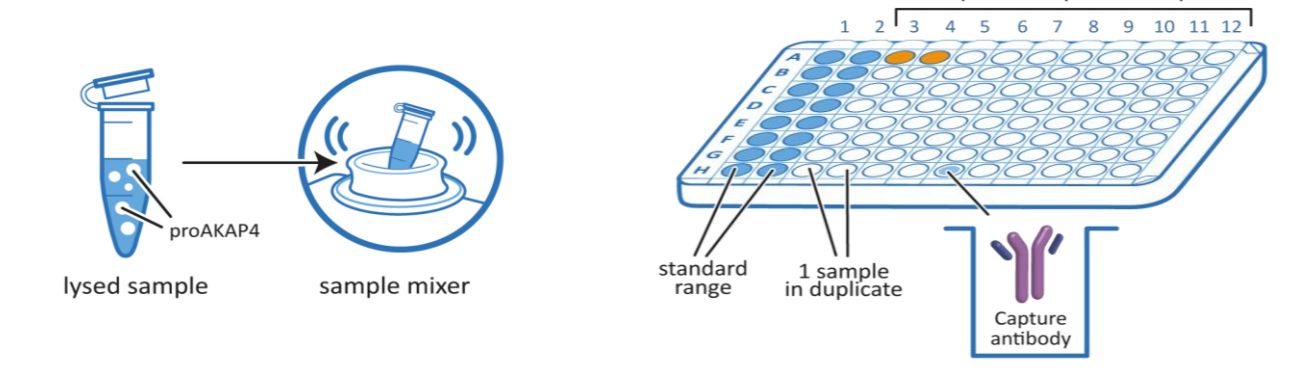
ProAKAP4 is expressed at the round spermatid stage



ProAKAP4 concentrations varies from a sperm to another. They are indicative of functional motility over time and how the sperm will remain motile up to the fertilization point.

### Principle of the 4MID® ELISA test to quantify proAKAP4 biomarker

To quantify proAKAP4, the spermatozoa are first lysed in a specific lysis buffers and then processed according to manufacturer's procedures.



In our study, we then evaluated the pertinence of the protein proAKAP4 concentrations as a read out of sperm quality in mouse models for high fat diet and cigarette smoking, with modulations under antioxidant treatments.

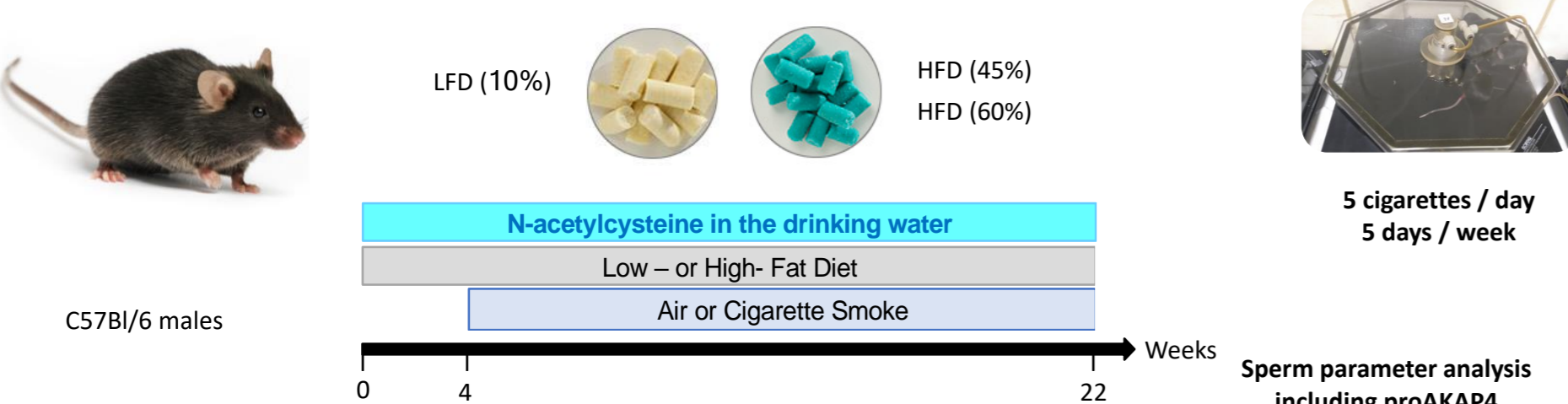
### Main Objectives

- Investigate the proAKAP4 sperm marker concentrations as a useful tool to:
  - Study the impact of environmental factors
  - Follow-up of therapeutic treatments
  - Analyze the recovery of good spermatogenesis

## Results

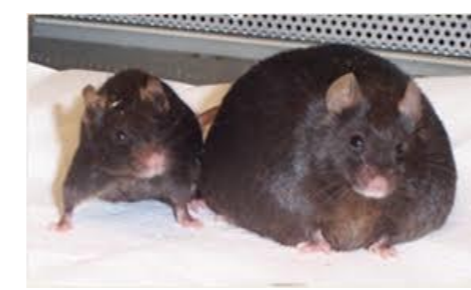
### Experimental Mouse Models of Unhealthy Behaviors

6 groups of mice (n=6 per group) were under low fat diet (LFD) or under two regimens of high fat diet (45 and 60% of lipids) and placed under normal or in cigarette smoke atmosphere (generated from 5 cigarettes per day, 5 days a week). Two groups in high fat diet regimens were treated or not with N-acetylcysteine.



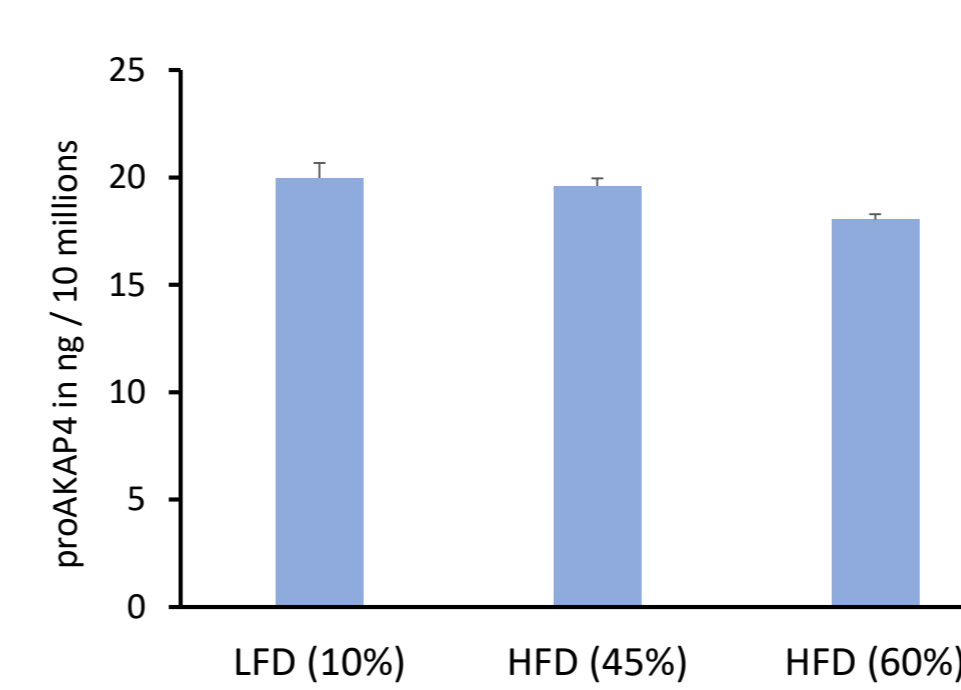
After 22 weeks, mice were sacrificed. Both testes were collected and spermatozoa were isolated from epididymis after percoll gradient.

In our mice models, regimens containing 45% or 60% of lipids lead respectively to overweight and obese mice, to model unhealthy behaviors.

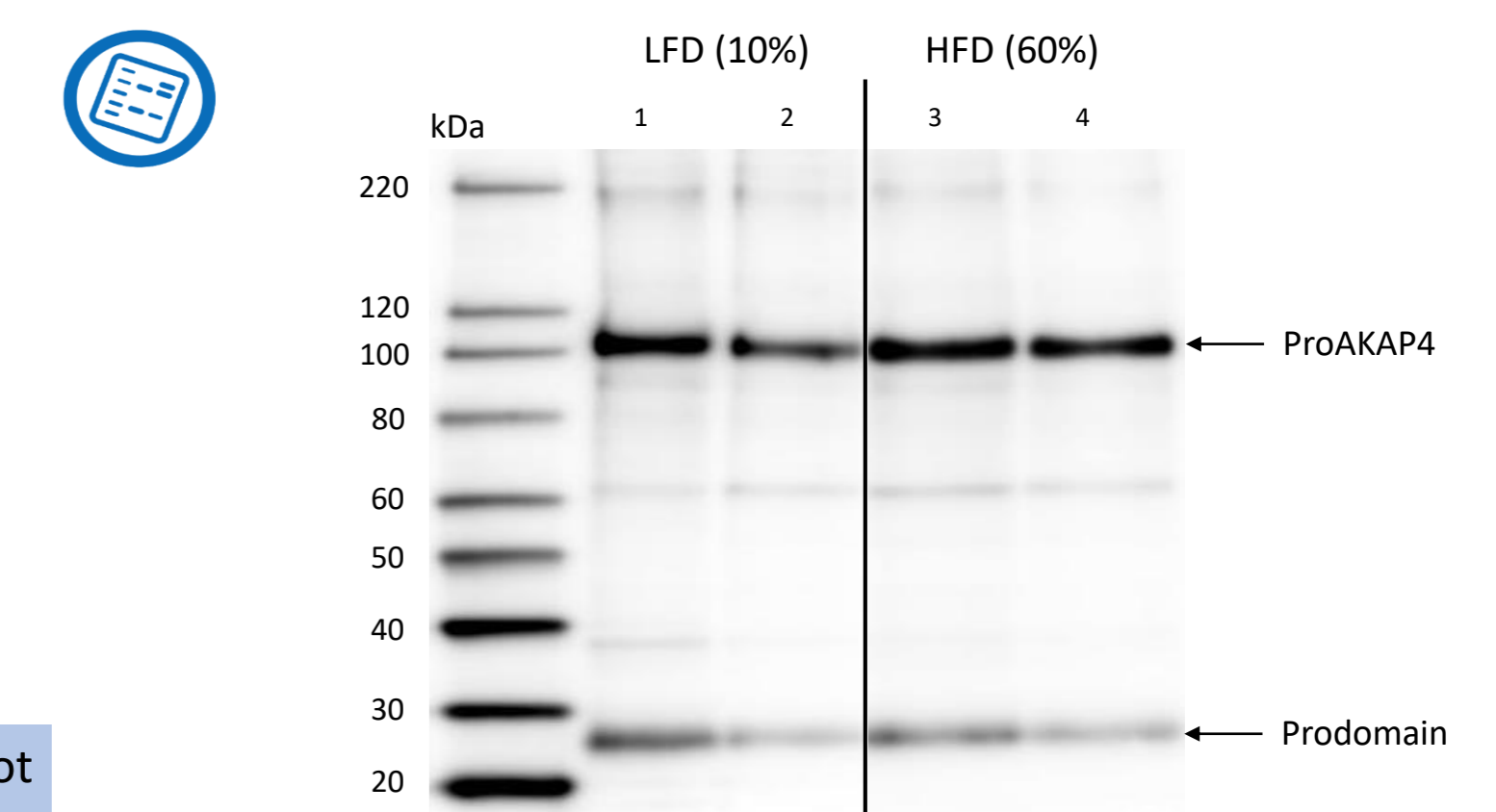


ProAKAP4/AKAP4 is a major and specific structural protein of the fibrous sheath of the principal piece of the flagellum (more than 50%). The ProAKAP4/AKAP4 was never released and/or not found in seminal plasma.

### ProAKAP4 concentration are not impacted by high fat diets, in overweight or obese mice



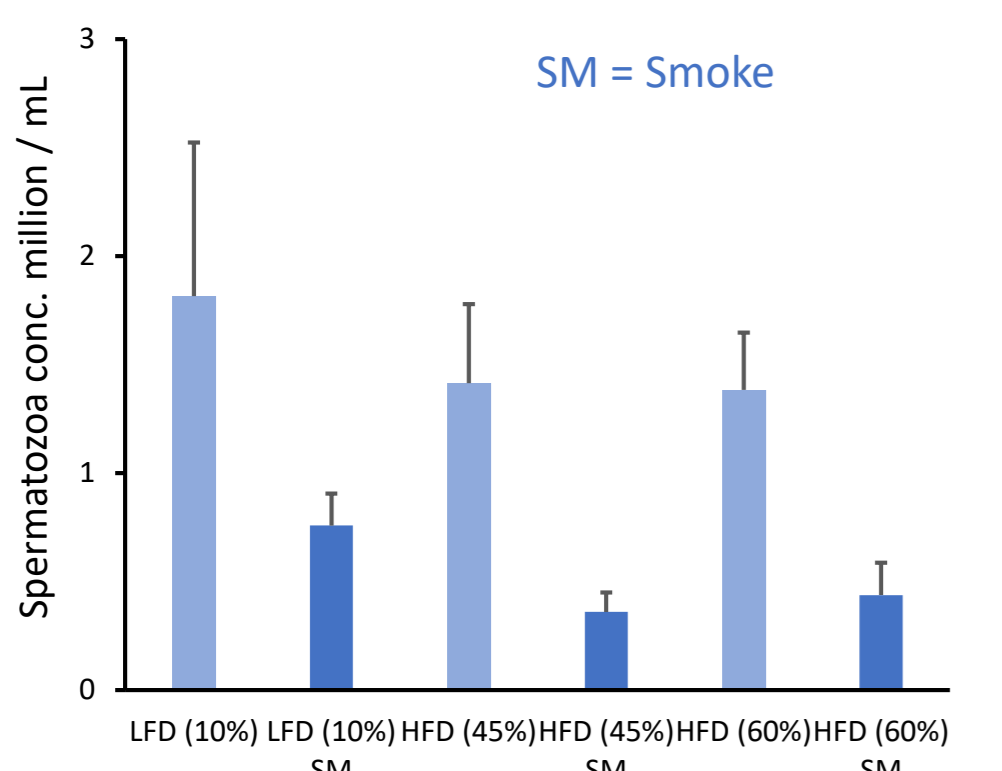
The concentrations of proAKAP4 in spermatozoa were not significantly different in the different regimens between the low fat diet (LFD) group and high fat diet 45% and high fat diet 60% groups, with levels comprised between 19.9 to 18.0 ng / 10 millions (n=6 per group).



No significant modification in proAKAP4 expression and metabolism were observed by western blotting using the 4BDX-1701 monoclonal antibodies (lanes 1, 2 : mice under LFD regimen, lanes 3, 4 : mice under HFD regimens).

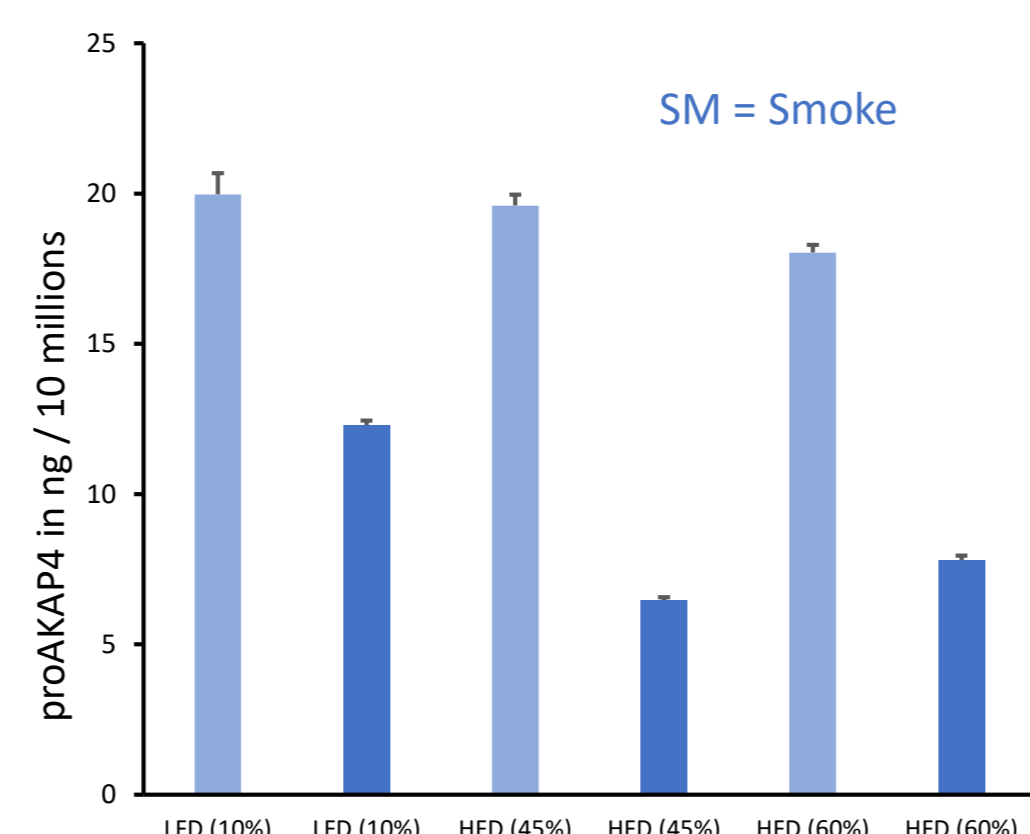
### ProAKAP4 expression enable to follow the impact of tobacco exposure on sperm quality

#### Production of spermatozoa is impaired by tobacco exposure



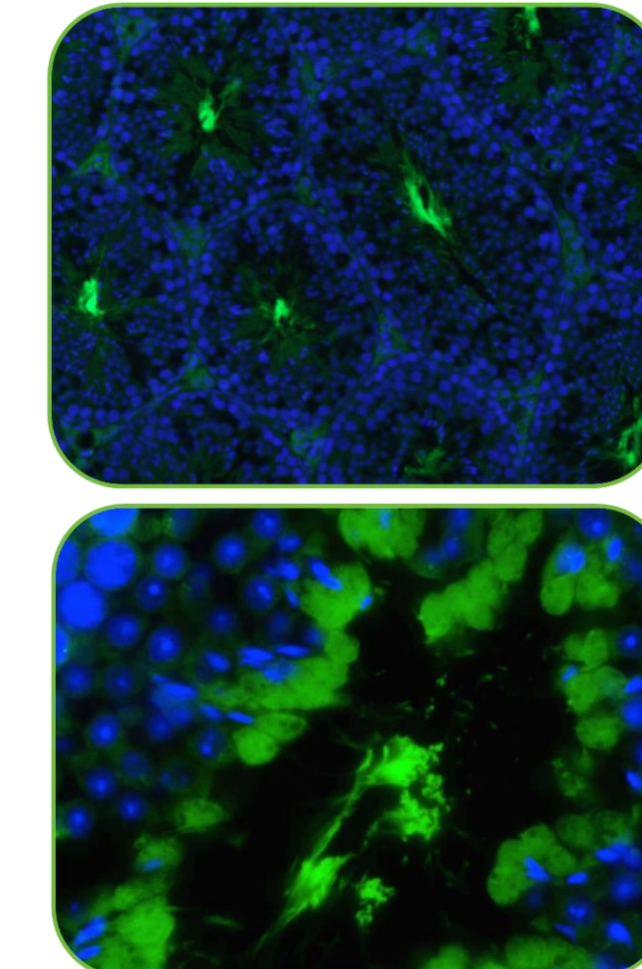
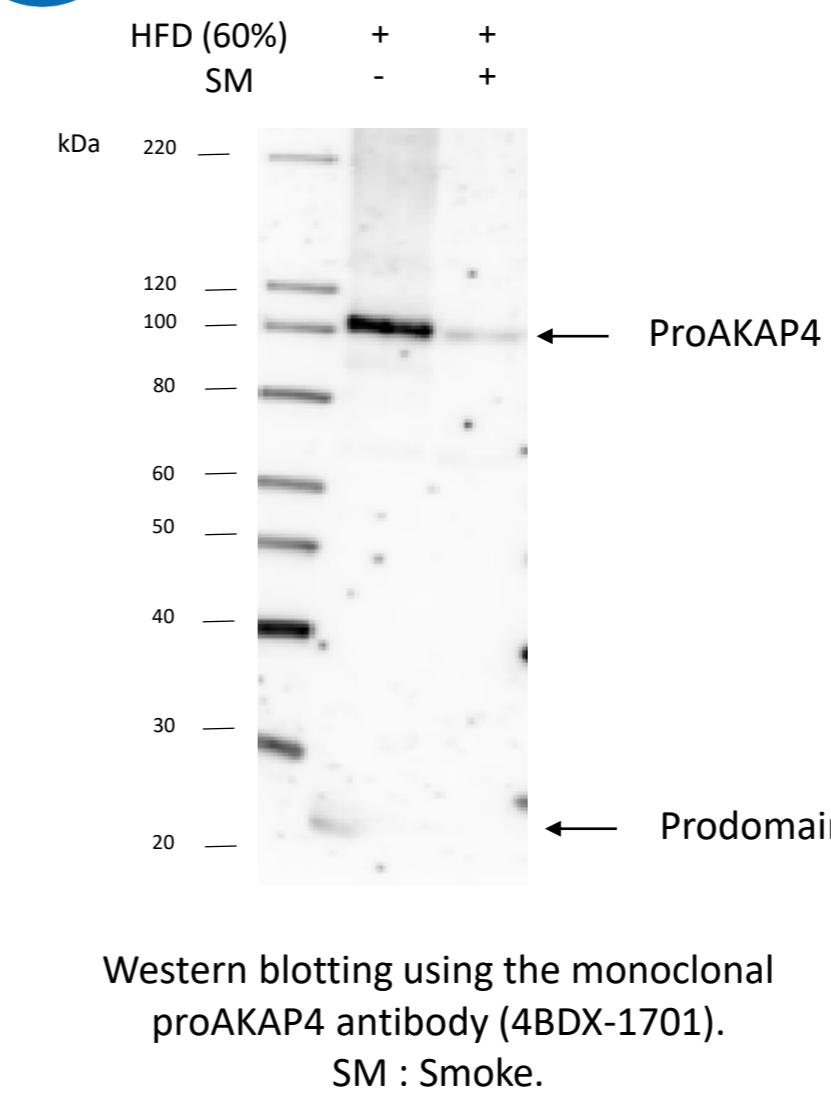
Tobacco exposure impairs significantly the rate of production of spermatozoa after 22 weeks of smoking exposure. This effect of tobacco is potentiated by HFD regimens (up to 2-fold factor).

#### ProAKAP4 loss of expression in mice exposed to tobacco



ProAKAP4 concentration is reduced from 19.9 to 12.3 ng / 10 millions of spz in mice the exposed to tobacco smoking atmosphere in LFD group (SM = Smoke). This reduction is more pronounced in the HFD regimens with a 2.5-fold reduction of the concentrations of proAKAP4 (from 18.0 to 7.4 in the HFD 45% regimen) and from 19.6 to 6.4 ng / 10 millions of spermatozoa in the HFD 60% regimen.

### ProAKAP4 expression and metabolism are impaired by tobacco smoke



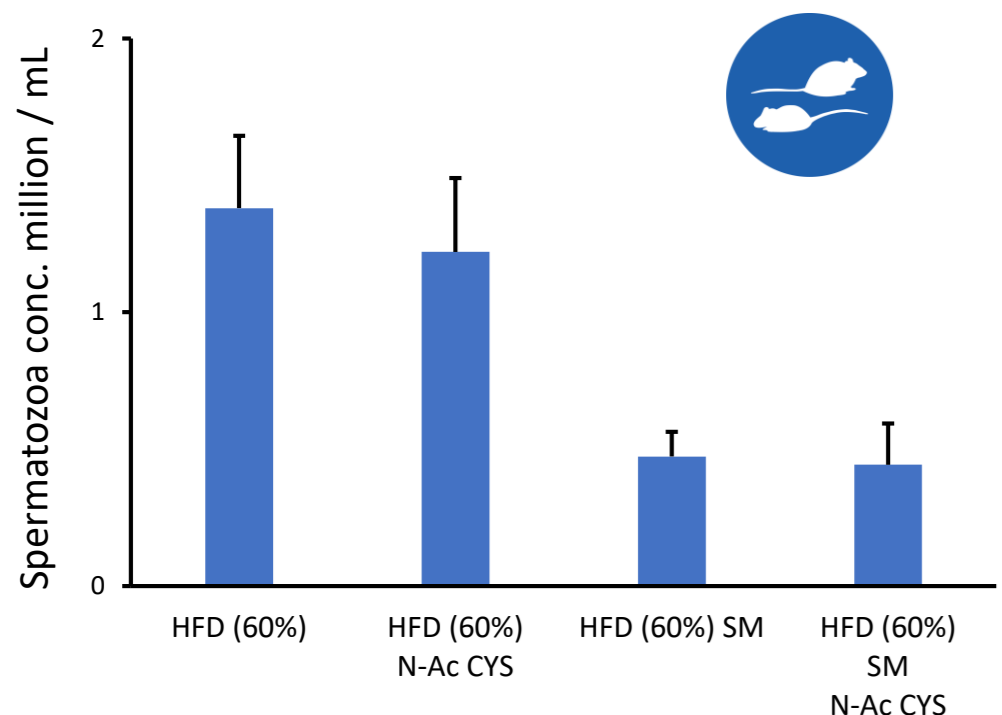
Sections of mouse testis (anti-proAKAP4, ref. 4BDX-1701)

**References**

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- Jumeau et al. (2018) *Andrology* 6(6): 854-859.
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- Sergeant et al. (2016) *Anim Reprod Sci.*, 169: 125-126.
- Sergeant et al. (2019) *Dairy Vet Sci J.* 11(1): 803-811.
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### ProAKAP4 expression is increased by antioxidant treatments

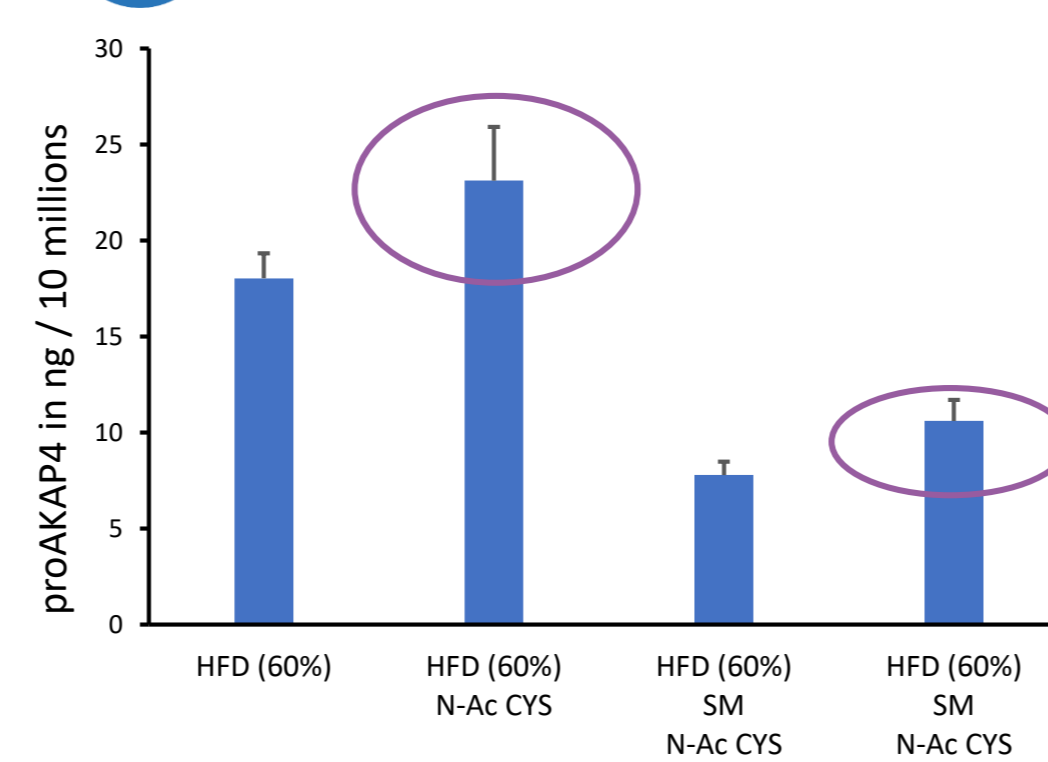
Treatment of male fertility mainly use antioxidants to improve sperm quality. Impact of tobacco smoking is principally acting through oxidative damage of sperm cell molecules.



N-acetylcysteine treatment does not improve spermatozoa production. The concentrations of spermatozoa were comprised between 1.8 to 0.43 millions per mL (n=6 per group) and significantly reduced in tobacco smoking groups by a 2.5 to 5.5-fold factor.

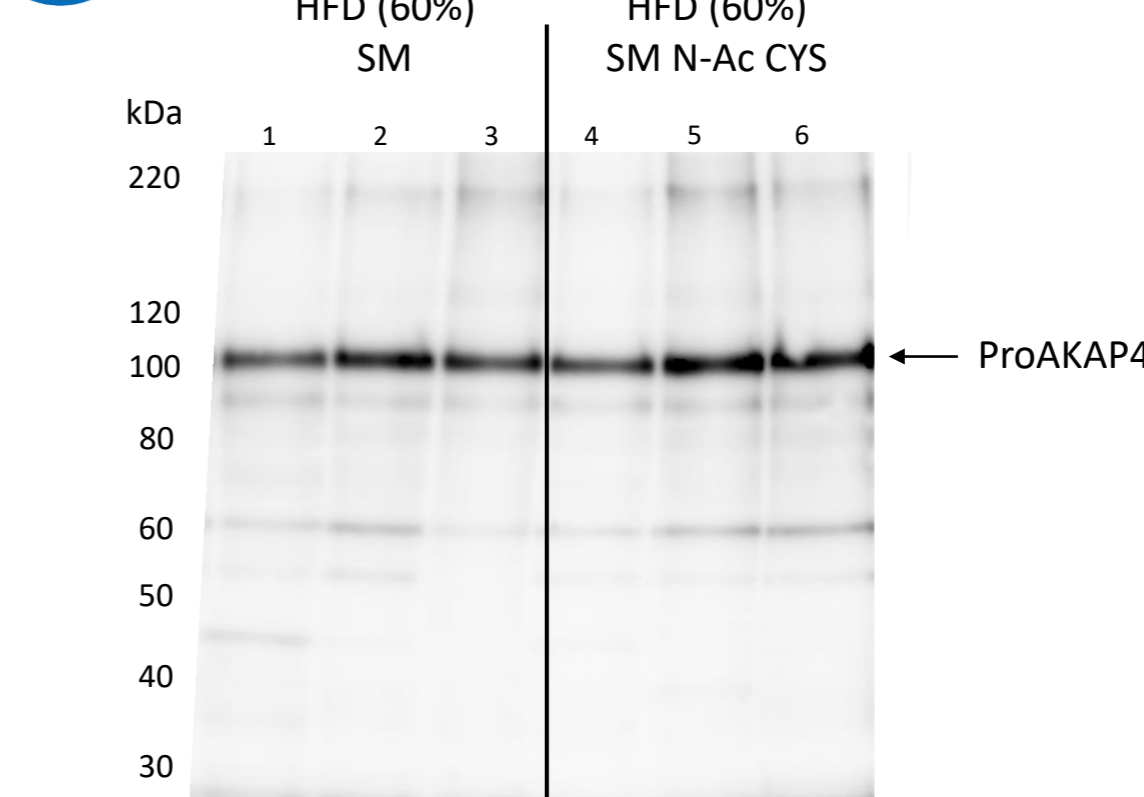
N-acetylcysteine treatments did not improve the concentration of spermatozoa in both high fat diet group and high fat diet plus tobacco exposure (SM = Smoke)

#### ProAKAP4 expression is improved by N-acetylcysteine treatment



ProAKAP4 concentration is increased from 18.0 to 23.1 ng / 10 millions of spz following N-acetylcysteine treatment in the HFD (60%) regimen (SM = Smoke). This increase is also observed in mice with the HFD (60%) regimen exposed to tobacco for which the treated with N-acetylcysteine increased proAKAP4 concentration from 7.7 to 10.6 ng / 10 millions of spermatozoa.

### ProAKAP4 expression and metabolism is improved by N-acetylcysteine treatment

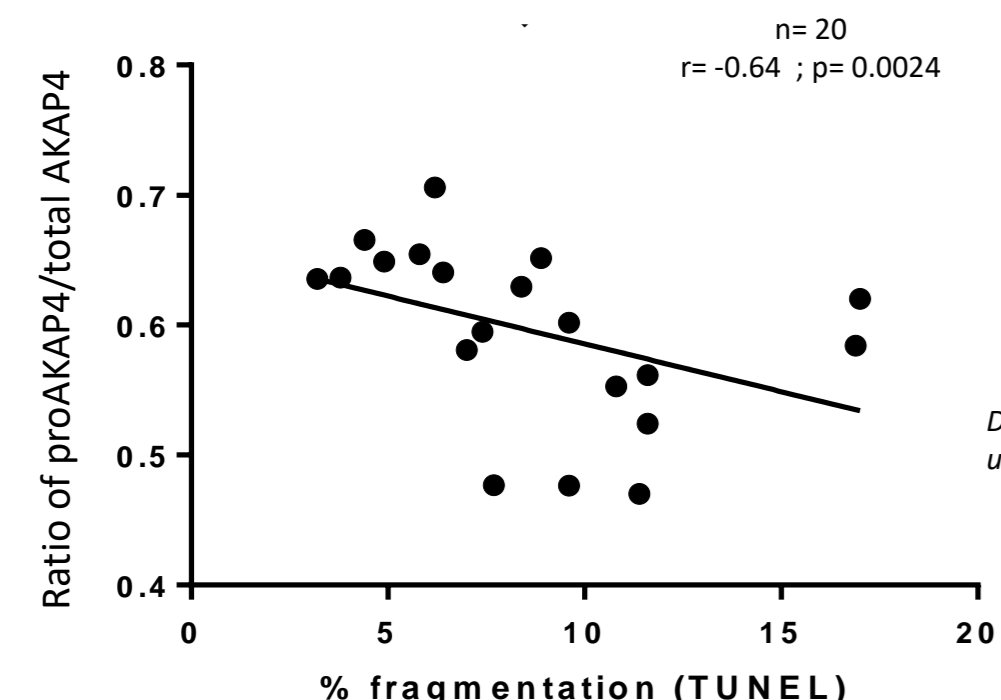


(lanes 1 to 3: mice under HFD and smoking; lanes 4 to 6 : mice under HFD, smoking and N-acetylcysteine treatment).

Both proAKAP4 expression and metabolism are improved in mice treated with N-acetylcysteine before exposure to tobacco and HFD regimen as observed by western blotting using the 4BDX-1701 proAKAP4 antibody.

Therefore, the stock of proAKAP4 sperm marker is preserved by antioxidant treatment to maintain sperm motility and fertility.

### ProAKAP4 concentrations as read-out of sperm quality in human sperm

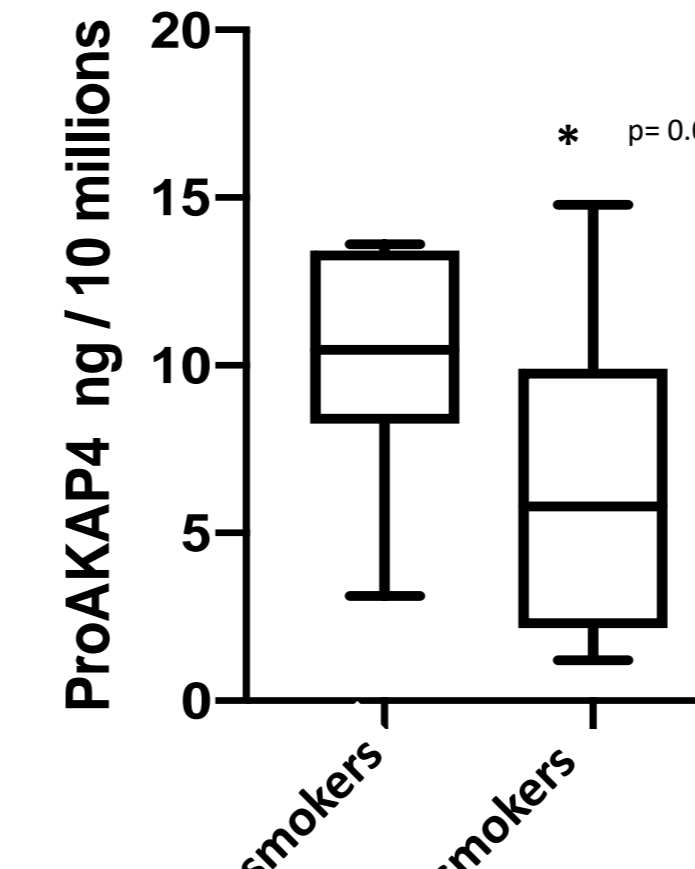


ProAKAP4 concentrations as measured using the Human 4MID® Kit were inversely correlated to the percentage of DNA fragmentation as assessed by TUNEL assay (In Situ Cell Death Kit Detection POD®, Roche France).

Men	Non smoker	Smoker
Number	n = 12	n = 17
proAKAP4 concentration in ng / 10 millions of spz	11.50	6.62

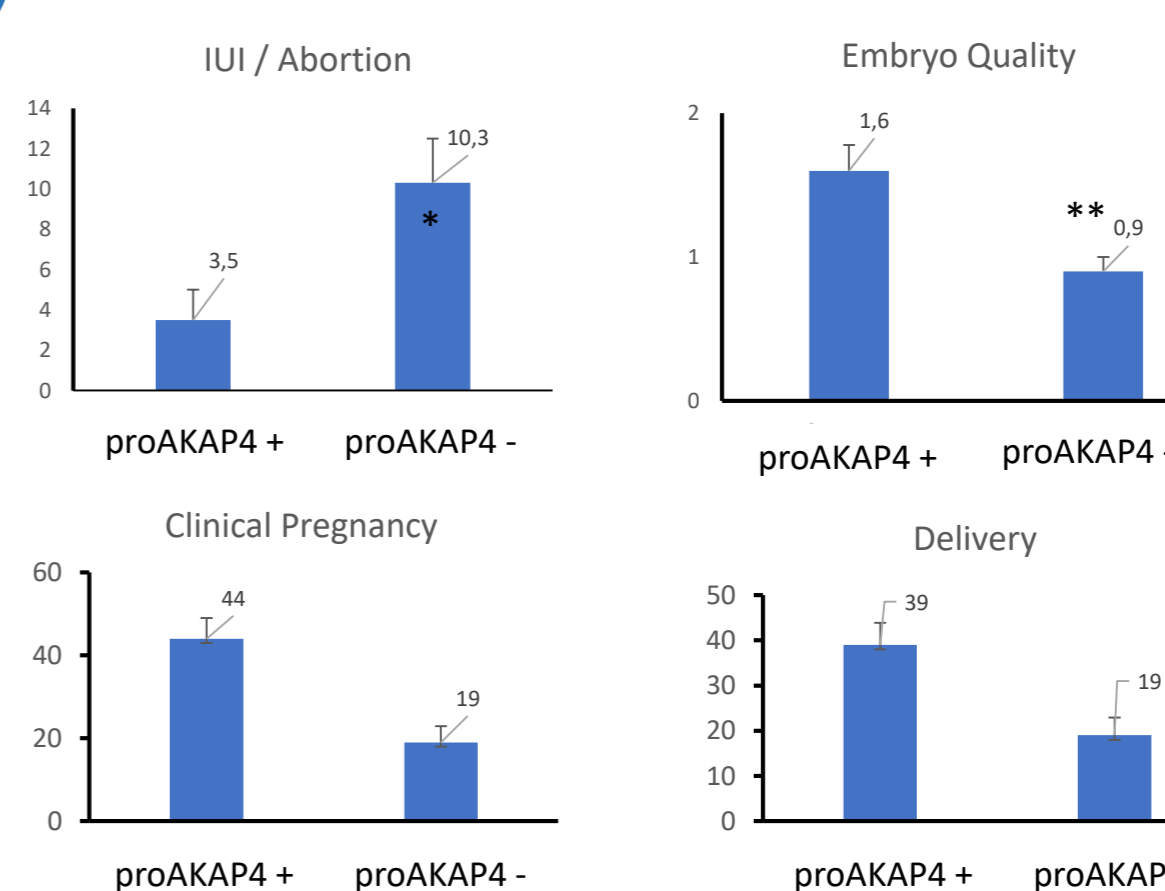
#### Loss of proAKAP4 concentrations in smokers compared to non-smokers

The concentrations of proAKAP4 were assessed using the human 4MID® Kit (ref. 4BDX-18K1, 4BioDx) in semen of 29 men consulting for couple infertility at the CHRU Lille (France). Smokers and non-smokers were confirmed by the quantification of cotinine in the seminal plasma using the ELISA Cotinine kit (Abnova).



The concentrations of proAKAP4 were markedly reduced in smokers when compared to non-smokers from 11.5 to 6.62 ng / millions of spermatozoa.

### ProAKAP4 concentrations in ART settings



Groups with proAKAP4, and without proAKAP4 expression were compared with IVF outcomes.

Preliminary clinical results show that proAKAP4 concentrations of proAKAP4 were shown to be correlated with:
 

- Reduced percentage of abortion in IUI (n=70)
- A better embryonic quality (n=70)
- An increase of global success rate in IVF/ICSI (n=70)

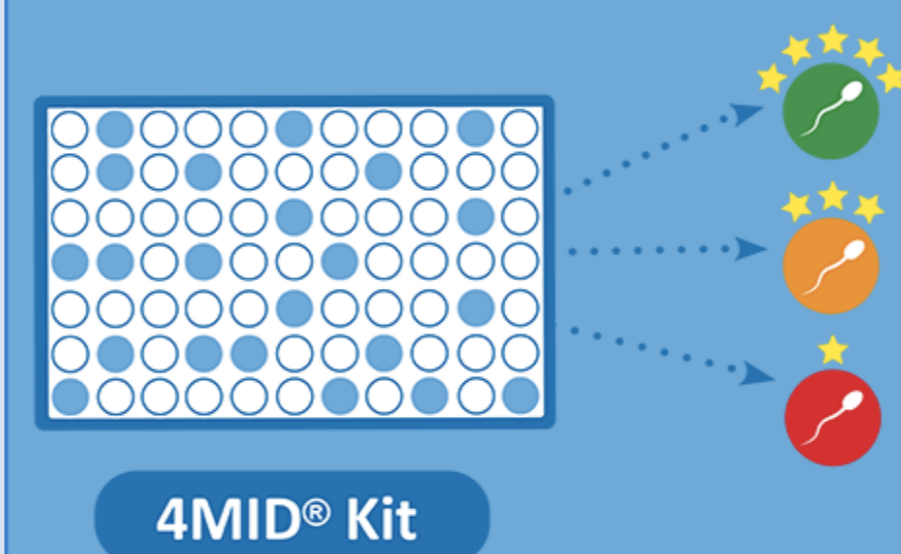
Sergeant et al. unpublished data

Therefore proAKAP4 concentration could be a good marker of embryonic quality and global success rate in IVF settings. However, further investigations in ART are needed to precise and validate proAKAP4 marker indications.

## Conclusions: proAKAP4 concentration as a new sperm parameter to follow sperm quality and fertility

Quantification of proAKAP4 concentrations is a valuable tool:

- To assess sperm quality and functionality
- To measure the effects of treatments (nutrients, antioxidants, vaccines, endocrine disruptors...)
- To assess environmental factor impacts
- To assess the recovery of a good spermatogenesis
- To distinguish between high, normal and subnormal sperm quality



The proAKAP4 marker is then a pertinent new sperm parameter to investigate in preclinical, toxicological or clinical studies needing the assessment of sperm quality under pathological and environmental conditions that impact male fertility.

Further investigations should be performed to evaluate the proAKAP4 variations as assessed by ELISA (as the 4MID® Kits) in antioxidative therapeutic approaches of male infertility.