Spermatech AS - making a male contraceptive by targeting a sperm specific protein kinase A

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WE HAVE IDENTIFIED and characterized the function of the protein CαS. CαS is a catalytic subunit of the protein kinase A (PKA) family of serine (Ser) and threonine (Thr) protein kinases. CαS is only identified one place in the body: in the sperm cell. CαS locates to the midpiece together with the energy generating mitochondria and in the tail. Using knock-out (KO) mice, we and others have shown that CαS regulates sperm cell motility and male fertility. Based on these findings the company Spermatech AS was founded in 2002. Spermatech AS is an early drug development company with the major goal of developing a male contraceptive based on targeting the CαS protein.

MALE VERSUS FEMALE CONTRACEPTION

Until now, women have taken the major responsibility for birth control and family planning. Contraceptives made for women are in most cases affecting fertility by targeting the endocrine system. This has shown to involve several side effects. Based on this knowledge, both hormonal and non-hormonal male contraceptives are being developed. Today, some male contraceptives are available for testing. Most of them indirectly affect sperm production and maturation by manipulating the male hormonal system. These contraceptives face the same challenges of harmful side effects as observed for female hormonal contraceptives. It is therefore a great need of new non-hormonal contraceptives with potentially less side effects.

Small scale clinical trials using male androgens are currently or soon to be enrolled. These rely on the use of hormones to suppress sperm development, e.g. by injecting testosterone into to the blood and thereby preventing gonadotrophin releasing hormone (GnRH) release from the pituitary gland by a negative feedback mechanism. GnRH is regulating the production of testosterone in the testis and lack of GnRH leads to lowered testosterone synthesis. As a consequence, these men have reduced fertility due to decreased sperm production and maturation but maintain secondary sexual male characteristics because of the injected testosterone. Unfortunately, these hormonal approaches must be administrated by injections or implants, and have severe side effects like atherosclerosis, aggression, acne and sleeping disturbances. An already established but non-reversible method is vasectomy (sterilization), which can be suitable for men who don't want to have (more) children.

HOW SPERM CELLS FERTILIZE THE EGG

A single human male ejaculation releases about

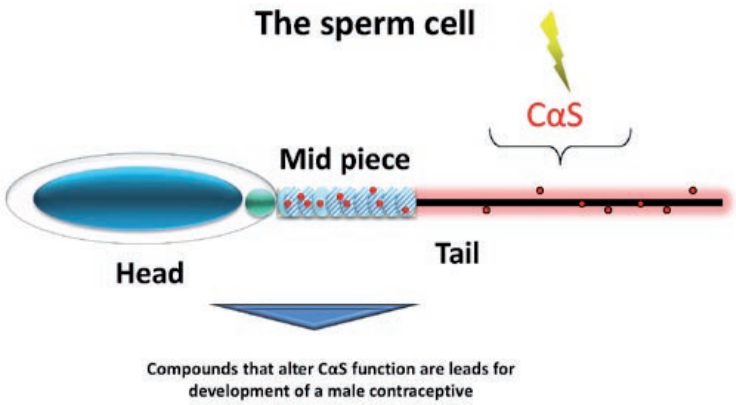


FIGURE 1. Sperm cells are specialized cells with a head containing the male DNA. Adjacent to the head is the midpiece which contains ATP generating mitochondria which are vital for sperm tail movement and thus male fertility. The catalytic subunit of PKA, CαS, is a protein uniquely found in the tail and midpiece of the sperm cell, and is pivotal for sperm cell motility.

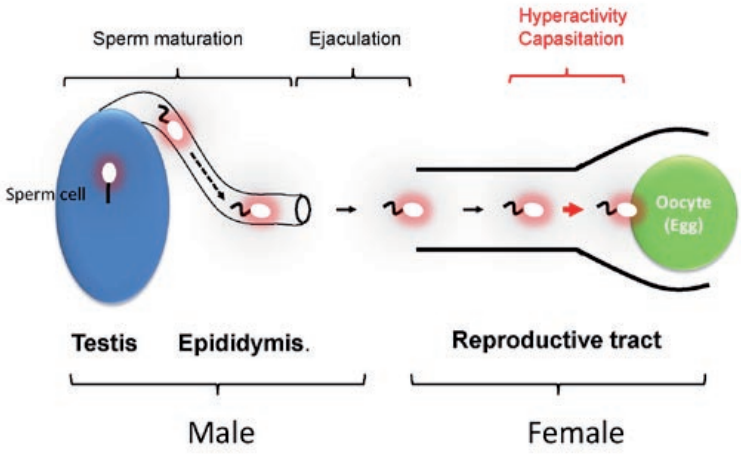


FIGURE 2. Sperm cells are formed and matured in the testis and *caput epididymis*. The sperm cells are activated to swim when stimulated by bicarbonate in the *cauda epididymis* before ejaculation. Sperm cells are prepared for fertilization of the egg in the female reproductive tract by undergoing the capacitation process.

2-5 mL of semen containing anywhere between 40 to 400 million sperm cells in total. If the sperm number is below 20 million cells per milliliter, a man is considered subfertile, and men with sperm counts below 3 million cells per milliliter are classified as infertile (The WHO manual for semen analysis). A single sperm cell (fig. 1) is about 40-50 microns long and carries 23 chromosomes. Sperm cells swim at about 20 centimeter per hour and can survive and stay fertile in the female reproductive tract for three to five days.

Sperm motility is inhibited in the seminal plasma but the cells will be gradually more active after entering the female reproductive tract. The tail (also called flagellum) spins like a boat propeller to move the sperm forward. This activity becomes even more profound when zeroing in on an egg. At this stage the sperm cells swim faster and their tail movements become more forceful and erratic. This behavior is called hyperacitivity and is one of the hallmarks of capacitiation. It is a process which prepares the sperm cell for breaking through two physical barriers that protect the egg from uncontrolled fertilization (fig. 2). The first barrier to the sperm is made up of so-called cumulus cells that encase the egg cell. The second barrier is a membrane called the zona pellucida. One of the proteins that make up the zona pellucida binds to a partner molecule on the sperm. This lock-and-key type mechanism is species-specific and prevents the sperm and egg from different species to fuse. Once a sperm reaches an egg's zona pellucida, it can partly dissolve the membrane with special enzymes. This event is called the acrosome reaction and completes the capacitation process. However, the enzymes are not sufficient for the sperm cell to completely break through and fuse with the egg alone. The sperm cell needs both the enzymes as well as being hyperactive in order to fertilize the egg.

CαS REGULATES MALE FERTILITY

Researchers at the University of Oslo identified in 2000 (Reinton et al. 2000) a protein which they designated CαS. Later characterization by the Oslo group as well as by two independent groups in the USA demonstrated that CαS is a splice variant of the catalytic subunit of protein kinase A (PKA) that is only expressed in sperm cells (Reinton et al. 2000; San-Agustin et al. 2000, 2001; Desseyn et al. 2000). Moreover,

FIGURE 3. Schematic drawing of the principle behind fragment library technology (left two figures) and HTS (right figure). It is considered simple to build a novel chemical entity from small fragments rather than modifying already existing entities in order to become a potent drug.

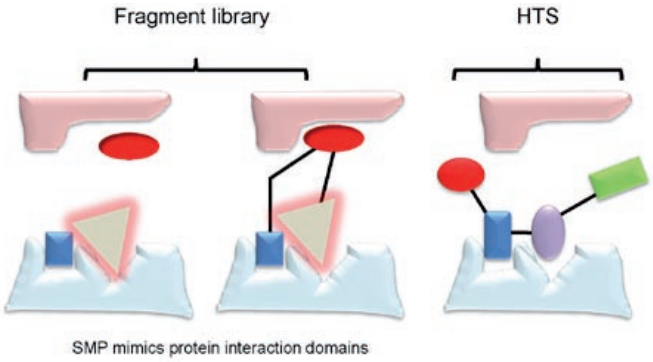
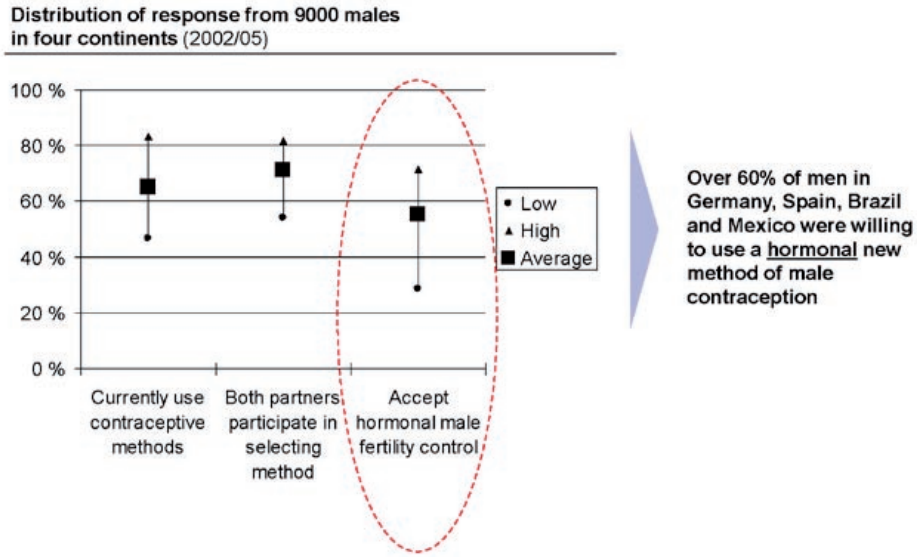


FIGURE 4. Men are willing to use contraceptives. Surveys made by the World Health Organization (WHO) conclude that over 50 % of men world wide are willing to take contraceptive if available. Moreover, over 60% of men in Germany, Spain, Brazil and Mexico were willing to use male contraception even though it is based on hormonal methods. Source: Heinemann, et al. (2005). "Attitudes toward male fertility control: results of a multinational survey on four continents."



CaS is the only catalytic subunit of PKA in sperm cells. Its expression is highly enriched in the tail as well as in the midpiece where it colocates with the mitochondria. By analyzing two genetically altered mouse strains, one carrying a targeted mutation of the whole Cα gene (Skålhegg et al. 2002) which encodes two splice variants of the Cα protein, Cα1 and CaS, whereas the other carrying a CaS specific ablation (knock-out, KO) (Nolan et al. 2004) it was demonstrated that CaS regulates sperm cell motility and male fertility**.** In these studies it was demonstrated that sperm cells isolated from CaS KO mice were unable to penetrate the egg unless zona pellucida was removed, suggesting that CaS ablation inhibits the capacitation processes. Moreover and most importantly, breeding experiments with such mice demonstrated that the male animals were 100 % infertile. Based on this documentation CaS has been stated as pivotal for sperm movement and male fertility. Hence, CaS represents a promising target for the development of a non-hormonal contraceptive.

SPERMATECH AS: AN EARLY DRUG DEVELOPMENT COMPANY

The identification of CaS as a key regulator of sperm capacitation has set the basis for establishing the company Spermatech AS (*http://www.spermatech.com/*). Spermatech AS was founded in 2002 by researchers at the University of Oslo and The Norwegian National Hospital (Rikshospitalet-Radiumhospitalet HF, R-R HF). Spermatech AS has a strong partnership with the University of Massachusetts Medical School, USA through Professor George Witman. Professor Witman is an expert in sperm cell biology and male reproduction and has been active in the field since the 1950s.

Spermatech AS is an early drug development company which focuses on the development of a male contraceptive based on the regulation of the CaS protein. As a first step in drug discovery, the 3-D structure of CaS has been determined by X-ray crystallography at the X-ray platform at R-R HF, (http://www.cmbn.no/group-bjoras.html). This has enabled Spermatech to set the

basis for computer/structure-based drug design, virtual screening, and high throughput screening (HTS). Using these methods the identification of potential CαS inhibitors (hits) will be performed in collaboration with Evotec AG (*http://www.evotec.com/en/*). Evotec is a world leading drug discovery company situated in Germany and England. To extend the way of targeting CαS function Spermatech has identified CαS binding partners by yeast two-hybrid screening. We are currently characterizing interaction domains and amino acids involved in protein-protein interactions. These amino acids represent hot-spots that give rise to so-called pharmacophores. Pharmacophore points represent the basis for virtual screening of small molecular probes (SMP) which will be used to disrupt CαS function.

The use of SMP to target key molecules known to be associated with physiological dysfunctions has proven successful in order to reduce symptoms and progression of medical disorders. SMPs are rational fundaments for the development and production of efficient medicines with a minimum of side effects. Specific SMPS are developed by systematic synthesis (medicinal chemistry) and are grouped into specialized libraries according to chemical and functional features. These compound libraries have been pre-screen using both virtual, chemical and biological methods. Based on huge downstream costs and previous high rate of failure in clinical trials, only the pre-screened libraries are used for HTS in chemical and/or biological assays to produce molecules that will bind to the target molecule (hits) (fig. 3). Hits represent chemical entities which may be developed into lead-like pre-drug candidates.

MEN ARE WILLING TO USE CONTRACEPTIVES

Recent interviews with men from four continents revealed that more than 50 % are willing to use contraceptives. In fact over 60% of men in Germany, Spain, Brazil and Mexico are willing to use male contraception even if it is based on hormonal methods (fig. 4). The acceptance of male contraceptives is growing and is expected to reach the same acceptance as today's female birth control pills when the major side effects have been decimated. This growing enthusiasm for male contraceptives reveals a huge market. This has caught the attention of "Big pharma" enterprises that have started huge efforts in developing male contraceptives.

NOVEL TECHNOLOGY

Spermatech's accomplishments, which may result in the development of a male contraceptive, have also provided a portfolio of technologies setting the basis for early drug discovery. This portfolio involves functional determination of proteins in genetically engineered animals and purification, characterization and 3-D structure determination of target proteins. This has enabled Spermatech to perform computer-based modeling and virtual screening, which further sets the basis for HTS and computer-based drug design. Thus, Spermatech represents a biotechnology-based industry which has generated novel competence and technology in drug determination in Norway.

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