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**Tereza Otčenášková**

Chemical communication of gametes  
Chemická komunikace gamet

Bachelor thesis

Supervisor: RNDr. Kateřina Hortová, Ph.D.

Consultant: doc. Mgr. Pavel Stopka, Ph.D.

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**Poděkování:**

Chtěla bych velmi poděkovat své školitelce paní RNDr. Kateřině Hortové, Ph.D. a konzultantovi panu doc. Mgr. Pavlovi Stopkovi, Ph.D. za vstřícnost, ochotu a cenné rady pro zpracování této bakalářské práce. Děkuji také svým rodičům za podporu nejen během celého studia.

**Prohlášení:**

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Podpis

**Abstrakt:**

Mnoho rozmanitých chemoatraktantů se podílí na směřování spermie k vajíčku, a to bez rozdílu, zda se jedná o živočichy s vnějším nebo vnitřním oplozením. Spermie jsou po uvolnění vystaveny různým faktorům, které jim poskytují nezbytné informace o jejich okolním prostředí. Tyto molekuly jsou strukturně velmi odlišné a prostřednictvím specifických receptorů, jenž jsou exprimovány na povrchu spermie, spouští rozdílné signální dráhy ovlivňující pohyblivost a regulující správnou funkci spermie. Celkové pochopení těchto mechanismů přináší zajímavé a často i překvapující úhly pohledu na evoluční vztahy mezi druhy bezobratlých a obratlovců. Některé principy, které byly považovány za přítomné výhradně u savců, jsou nyní odhalovány i u mořských bezobratlých a naopak. Stávající znalost interakcí mezi savčími gametami je hojně využívána obory medicíny spojenými s reprodukcí. Výzkumy zaměřené na toto téma tak pomáhají objasnit mnohé příčiny neplodnosti, stejně jako vyvíjet nové druhy antikoncepce či zvyšovat úspěšnost umělých oplodnění.

Klíčová slova: chemotaxe spermií-vajíčka, CatSper kanály, chuťové receptory, formyl peptidové receptory, progesteron, atriální natriuretický peptid

**Abstract:**

Many diverse chemoattractants play a part in sperm guidance towards the oocyte without any difference whether organisms with external or internal fertilization. Upon release, spermatozoa are exposed to various cues providing essential information about their surrounding environment. These molecules are structurally very distinct and they induce via specific receptors, expressed over the sperm cell surface, different signalling pathways influencing the sperm motility and regulating the correct sperm function. The overall understanding of these mechanisms brings interesting and often unexpected points of view on evolutionary relationships between invertebrate and vertebrate species. Some principles believed to be shown exclusively only by mammals are nowadays revealed also by marine invertebrates and vice versa. The current knowledge of human gametes interactions is used in fields of medicine that are connected to the reproduction. Thus, with a help of current knowledge, researches aim to shed more light into causes of infertility, as well as to develop novel contraceptives or enhance the rate of successful in vitro fertilizations.

Key words: sperm-egg chemotaxis, fertilization, CatSper channel, taste receptors, formyl peptide receptors, progesterone, atrial natriuretic peptide

**List of abbreviations:**

ANP ... atrial natriuretic peptide

ATP ... adenosine triphosphate

cAMP ... cyclic adenosine monophosphate

CatSper ... cation channel of sperm

cGMP ... cyclic guanosine monophosphate

CNG ... cyclic nucleotide-gated channel

DAG ... diacylglycerol

FPR ... formyl peptide receptor

GC ... guanylyl cyclase

GPCR ... G protein-coupled receptor

GTP ... guanosine triphosphate

IP3 ... inositol triphosphate

OOC ... oocyte-cumulus complex

OR ... odorant receptor

PKA ... protein kinase A

PLC ... phospholipase C

RANTES chemokine ... regulated on activation normal T-cells expressed and secreted chemokine

SAAF ... sperm-activating and -attracting factor

sAC ... soluble adenylyl cyclase

SAP ... sperm-activating peptide

sNHE ... sperm-specific voltage-dependent  $\text{Na}^+/\text{H}^+$  exchanger

T1R (Tas1R) ... taste receptor type 1

T2R (Tas2R) ... taste receptor type 2

tmAC ... transmembrane adenylyl cyclase

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## 1. INTRODUCTION

The process of fertilization is a key event for the beginning of a new organism. Before this happens, the contact of male and female gametes is necessary. Spermatozoa of both invertebrate and vertebrate species, therefore, have to possess the ability to sense and respond to diverse chemical molecules according to which sperm orientate. These molecules are called chemoattractants and the action of the orientated movement up their gradient is known as chemotaxis. The overall mechanism of this sperm guidance is based on fluctuation in intracellular concentration of calcium ions ( $[Ca^{2+}]_i$ ), in sperm flagellum leading to different motility patterns (Harper & Barratt 2007).

To identify the true chemoattractant is not so simple, as the sperm accumulation – one of the criteria of sperm chemotaxis – can be caused also by chemokinesis or sperm trapping (Eisenbach 1999). Thus, using of special assays is required for distinguishing the real chemotactic response. Similarly, the identification of attractant origin is also problematic. Up to date, only a few chemoattractants have been confirmed. In water, for invertebrates with external fertilization, the main secreting source of species-specific peptides is the jelly layer surrounding oocyte (Ward et al. 1985). On the other hand, in vertebrates some physiological attractant sources have been found in follicular fluid (Ralt et al. 1991), cumulus cells and the oocyte (Sun et al. 2005), and they form together the so-called oocyte-cumulus complex (OCC). Especially in mammals, because of their internal fertilization, it is complicated to mimic a unique environment inside the female genital tract in *in vitro* experiments. Also, the oestrous cycle causes changes which cannot be negligible and strongly influences that environment. It is therefore a question of whether observations seen in experiments *in vitro* can also be observed *in vivo*. Besides chemotaxis, mammalian spermatozoa are also guided by thermotaxis - sensing and responding to temperature gradient (Bahat et al. 2003) and rheotaxis – movement against a fluid flow (Mikki & Clapham 2013).

The aim of this thesis is to summarize the current knowledge about the mechanisms of chemical communication of gametes in a sense of chemotaxis prior to the gamete fusion. The characterization of molecular mechanisms and cellular signalling pathways involved in a sperm guidance towards the egg will be described, same like the general comparison between invertebrate and vertebrate species with a special focus on mammals. This work will also mention points which, despite the decades of intensive research, still remain unclear and wait to be discovered.

## 2. CHEMOTAXIS IN MODEL INVERTEBRATE AND NON-MAMMALIAN VERTEBRATE SPECIES

### *2.1. Basic differences and similarities*

Sperm chemotaxis preceding fertilization is a widespread phenomenon. First attractants were discovered in the second half of the 20<sup>th</sup> century (Brokaw 1958) and since that time our evidence has grown a lot. Even though it could seem that sperm chemotaxis has among different species the universal manifestation, it is not completely true.

The most significant difference between marine invertebrates and mammals consists in species specificity. It is mainly based on a different way of the fertilization. As marine spermatozoa are spawn into aqueous environment which contains sperm and eggs from many distinct species, the high species-specific interactions between gametes (Miller 1985) are necessary for preventing cross-species fertilization (Eisenbach & Giojalas 2006). On the contrary, it could seem that mammalian sperm could lack the species specificity of chemotactic response due to internal fertilization. Some studies even proved this statement. For instance, experiments with the follicular fluids of cows, rabbits and humans and their spermatozoa showed no species specificity (Sun et al. 2003). However, another study did not prove the same reply to odorants (for more information about the role of odorants in chemotaxis see chapter 4.2.) in human and murine sperm (Fukuda et al. 2004). Taken this together, the species specificity in sperm chemotaxis occurs in both water invertebrates and vertebrates but it seems to have a bigger importance, in the sense of averting the cross-species fertilization, in marine species.

Another important aspect is a responsiveness of spermatozoa to chemoattractant. In marine invertebrates, a very large fraction of the sperm population exhibit chemotactic behaviour caused by the attractant (Miller 1985). It is suggested that it increases the recruitment of sperm so that the chances of sperm reaching the eggs are therefore higher (Eisenbach & Giojalas 2006). In the contrast, only a small fraction of the mammalian sperm population chemotactically responses to attractants. These spermatozoa are matured through the process of capacitation, which is represented by a series of chemical and molecular changes and gives sperm the fertilizing ability. Capacitation is a transient stage and sperm occur in this phase only once over a species-specific time period resulting in selection of fertilizable spermatozoa (Cohen-Dayag et al. 1994, 1995).

Both groups share the necessity of sperm chemotaxis. In the spacious water environment, spermatozoa cannot count on the coincidental meeting with eggs (Miller 1985). Similarly, in mammals including humans, the sperm guidance is essential as well (Eisenbach & Tur-Kaspa

1994), although it could be expected, due to restricted size of female genital tract where semen is directly ejaculated, that sperm would not need any leading. Thus, the long-time believed dogma that spermatozoa can reach the egg by pure coincidence has been rejected (Eisenbach & Giojalas 2006).

As already mentioned above, chemotaxis is characterized as a directed movement in the concentration gradient of chemoattractant and in sperm is modulated by intracellular calcium ions (Harper & Barratt 2007) which is generally common in both invertebrate and vertebrate species. The diverse sperm behaviour reflects dynamic changes in flagellar movement such as different beating velocity, frequency, symmetry and amplitude.

## 2.2. Description of chemotaxis in marine invertebrates

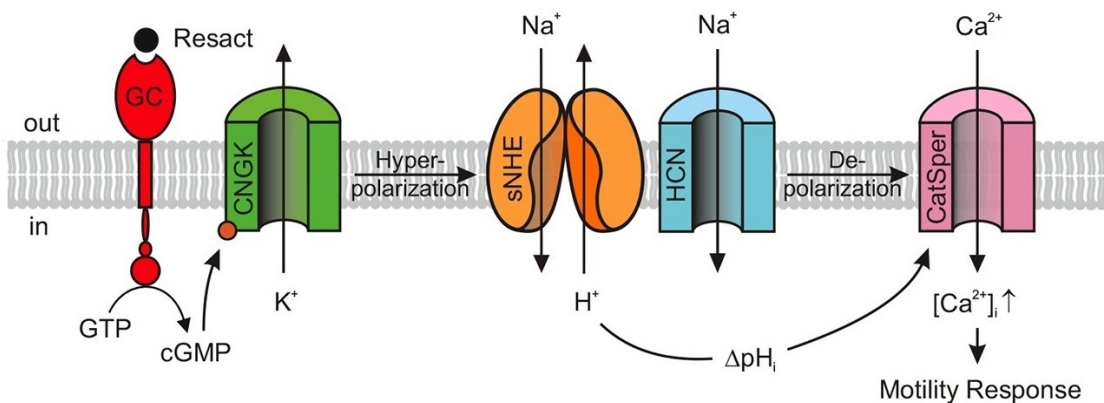
Marine invertebrate species are commonly used as a model organisms for many kinds of research and chemotaxis-related studies are not an exception. First egg-derived, species-specific chemoattractant called speract was identified in sea urchin *Strongylocentrotus purpuratus* (Hansbrough & Garbers 1981). Speract belongs to the family of sperm-activating peptides (SAPs). It is noteworthy that later study showed the true chemotactic response to speract in different sea urchin *Lytechinus pictus* spermatozoa (Guerrero et al. 2010).

Another example of SAP is resact, a 14-amino acid peptide specific for sea urchin *Arbacia punctulata* (Ward et al. 1985). Their secretion from the egg jelly layer starts after the completion of the second meiotic division (Miller 1985) (in this stage sea-urchin oocytes are fertilized), which ensures besides the species specificity also the temporal specificity. SAP can also be found in starfish, namely asterosap attracting *Asterias amurensis* sperm (Böhmer et al. 2005).

The SAP-induced signalling pathway (see Fig. 1) is based on a guanylyl cyclase (GC) located on the sperm flagellum (Matsumoto et al. 2003). The receptor is either a transmembrane GC (tmGC) that is in case of resact (Singh et al. 1988) and asterosap (Nishigaki et al. 2000) or it associates with GC same like the receptor for speract (Bentley et al. 1988). The peptide-binding to its receptor activates the catalytic domain synthesising cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP). The elevation of the level of cGMP causes the opening of cyclic nucleotide-gated potassium channels (CNGK) and the efflux of  $K^+$  ions leads to membrane hyperpolarization (Galindo et al. 2000). This, in turn, opens hyperpolarization-activated, cyclic nucleotide-gated (HCN) sodium channels and together with the sperm-specific voltage-dependent  $Na^+/H^+$  exchanger (sNHE) leads to  $Na^+$  influx and intracellular alkalinisation (Lee & Garbers 1986; Nishigaki et al. 2001). The



increase in intracellular pH likely activates soluble adenylyl cyclase (sAC) synthesising from adenosine triphosphate (ATP) cyclic adenosine monophosphate (cAMP) (Nomura et al. 2005). Finally, membrane depolarization results in influx of  $\text{Ca}^{2+}$  ions through membrane calcium channels (González-Martínez et al. 1992). As reported recently, sea urchin spermatozoa control the calcium influx via unique sperm-specific CatSper channels (Seifert et al. 2015) previously identified only in mammalian sperm (Ren et al. 2001). This discovery dismantled our conviction that CatSper channels were developed only in evolutionary higher species such as mammals.



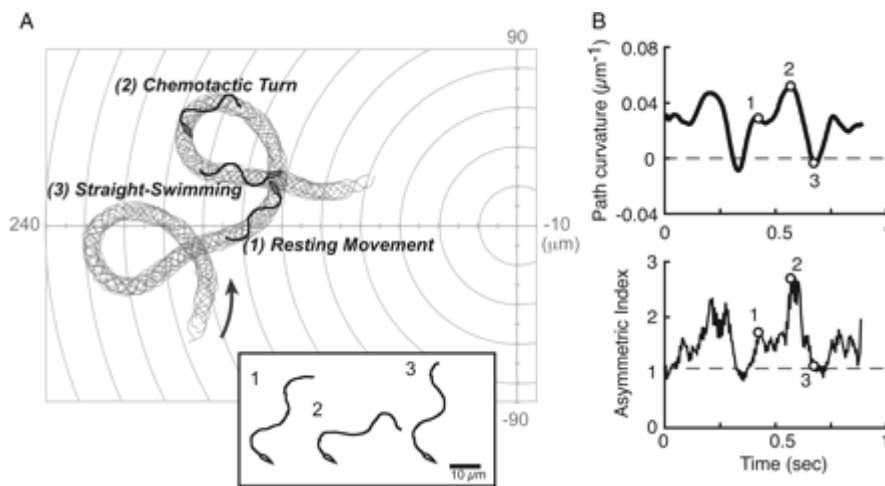
**Figure 1:** Resact-induced signalling pathway in *A. punctulata* sperm flagellum: After binding of resact to its receptor, the triggered signalling cascade leads to hyperpolarization of membrane,  $\text{Na}^+$  influx following by increase in intracellular pH and results in elevation of  $[\text{Ca}^{2+}]_i$ . The participating transmembrane molecules are guanylyl cyclase, CNGK potassium channel,  $\text{Na}^+/\text{H}^+$  exchanger, HCN sodium channel and CatSper channel. For clarity sAC synthesising cAMP is not displayed there. (Taken from Seifert et al. 2015).

Sea urchin spermatozoa in the absence of chemoattractant exhibit circular movement with low asymmetric flagellar beating. After exposure to attractant gradient, sperm response biphasic: first, they show “turn and run” motility pattern characterised by alterations of  $[\text{Ca}^{2+}]_i$  (Guerrero et al. 2010) and by increase and decrease in flagellar curvature during turns and straight movement, respectively (Kaupp et al. 2003), and second, spermatozoa restore their circular swimming regime. Interestingly, diameter of these circles is larger than before stimulation and gradually diminishes to the original size (Böhmer et al. 2005). The resuming of circular movement is thought to be caused due to degradation of cGMP by active phosphodiesterase (PDE) (Kaupp et al. 2003).

Chemoattractants have been determined also in another marine species. For example, spermatozoa of an ascidian *Ciona intestinalis* are governed by egg-released sperm-activating and -attracting factor (SAAF) which is structurally a sulphated steroid (Yoshida et al. 2002). As it is mentioned in its name, SAAF has two different functions. First, as ascidian spermatozoa released into water are either immotile or slightly motile, the SAAF-induced

Ca<sup>2+</sup> entry causes hyperpolarization following by cAMP increase (Izumi et al. 1999). cAMP-activated protein kinase A (PKA) phosphorylates axonemal proteins resulting in activation of basic motility (Nomura et al. 2000). Second, the chemotactic response is ensured by increase of flagellar beat frequency and Ca<sup>2+</sup>-dependent changes in flagellar waveform which is caused by sAC-synthesised cAMP. To sum it up, there are two distinct adenylyl cyclases involved – transmembrane AC (tmAC) playing a role in sperm motility activation and sAC regulating chemotactic movement, respectively (Shiba & Inaba 2014). Thus, the signalling pathway modulating the sperm chemotactic response in both sea urchins and ascidians seems to share the sAC-involving part.

Without the presence of attractant gradient, activated spermatozoa move in circles displaying constant asymmetric beating of flagellum (Yoshida & Yoshida 2011). When sperm are exposed to SAAF gradient, the chemotactic behaviour is governed by dynamic switching of two motility patterns resulting in a spiral path: first, chemotactic turn caused by increase in flagellar waveform asymmetry leading to an increase of path curvatures (Yoshida et al. 2002) and second, straight movement with symmetric beating and low path curvatures (Shiba et al. 2008) (see Fig. 2). Ascidian spermatozoa exhibit this chemotactic turn also during the perception of descending the SAAF gradient (Yoshida et al. 1993).



**Figure 2:** Switching of different motility pattern in the *C. intestinalis* spermatozoa: (A) Changes in the movement paths – the resting movement similar to the one observed without the presence of SAAF (1), the chemotactic turn as a result of swimming away from the attractant (2), the straight movement following the turn (3). On the inset image are shown traces as they respond to different flagellar beating. (B) Changes in the path curvature and asymmetry of beating. (Taken from Yoshida & Yoshida 2011; modified from Shiba et al. 2008).

Certain analogy of SAP, found having a role in sea urchin sperm guidance, also possesses the attractant of spermatozoa of the cuttlefish *Sepia officinalis*. This C-terminally amidated hexapeptide was for its origin called SepSAP, responding to *Sepia* sperm attracting peptide

(Zatylny et al. 2002). In marine molluscs, another chemoattractant has been specified as well. The amino acid tryptophan was shown to be sufficient for promoting the sperm recruitment to eggs of red abalone *Haliotis rufescence*. Noteworthy is that the chemotactic response was observed only to the L-isomer of tryptophan indicating the stereospecificity of sperm response (Riffell et al. 2002). Also, a molecule with the alcohol structure has been identified as a sperm chemoattractant, concretely an egg-derived unsaturated fatty alcohol, doceda-2,4-diynol, attracting spermatozoa of the coral *Montipora digitata* (Coll et al. 1994). In summary, this short list of chemoattractants and their signalizations among different marine invertebrate species is of course not complete and only confirms that, despite the many years of researches, our knowledge is still limited. However, it can be concluded that chemoattractants play an important role in initiating of progressive motility and in changing of motility pattern.

### 2.3. Brief description of chemotaxis in non-mammalian vertebrates

To characterize the mechanism of sperm chemotaxis, which is so complex and diverse at the same time, among all vertebrate species is out of reach of this thesis. Therefore, the whole next chapter relates to evidence about chemotaxis only in mammalian species.

The most studied non-mammalian vertebrates are amphibians with *Xenopus laevis* as a frequent model organism. Also in its case, sperm chemoattractant has been identified. Acidic protein allurin (Olson et al. 2001) is secreted by the egg jelly coat after hydration upon spawning as already observed before (Al-Anzi & Chandler 1998). Allurin has the homologous sequence with the mammalian cysteine-rich secretory protein (CRISP) family (Olson et al. 2001), whose members are known for sperm-binding ability during spermatogenesis (Kratzschmar et al. 1996). According to phylogenetic analysis, current believe is that an ancestral protein of this protein family has given rise to allurin as well (Olson et al. 2001). Later study showed allurin-elicited chemotactic response also in *X. tropicalis* sperm indicating that allurin causes heterospecies chemotaxis (Burnett et al. 2008). Molecular details are hardly known; however, a recent study indicates that the sperm motility is activated when spermatozoa are ejaculated into low osmotic water. Hypotonicity, then, leads to synthesis of cAMP by tmAC resulting in activation of cAMP-dependent PKA and in turn protein phosphorylation (O'Brien et al. 2014). Chemical communication between gametes of other non-mammalian vertebrates has not been studied extensively so far, thus, it is not mentioned here.

### 3. PROCESSES PRIOR TO MAMMALIAN FERTILIZATION

In order to proceed towards chemical communication of mammalian gametes, the basic summary of events that gametes undergo prior to their binding and fusion needs to be addressed. Mammalian species are known for their internal fertilization. As sperm are placed either in the vagina (for example in primates, dogs and rabbits) or in the uterus (in rodents or pigs) (Harper 1982), they have to reach the part of the Fallopian tube where fertilization takes place named ampulla. However, to make this happens, there are some processes which sperm are required to undergo at first. Despite the large number of ejaculated spermatozoa, only a small portion of them successfully enter the oviduct (Suarez et al. 1997). Besides the elimination by the female genital tract, the critical step is also the passing through the utero-tubal junction (Tokuhiko et al. 2012). Once this is managed, spermatozoa of most mammalian species are attached to the ciliated epithelial cells (Suarez 2002) of the isthmus of oviduct which functions as a sperm storage site (Harper 1994). The molecules involved in this interaction are diverse among species but can be generalized as carbohydrates found in the epithelial cells and lectin-like proteins expressed on spermatozoa (Suarez 2002).

During this attachment, spermatozoa undergo the process of capacitation known as the set of physiological changes which make the sperm able to fertilize the oocyte (Austin 1951; Chang 1951). The first change is the membrane modification. The cholesterol removal by serum albumin presented in the oviductal fluid leads to increase of membrane fluidity and permeability for ions (Davis et al. 1980) and to clustering of lipid rafts containing zona pellucida-binding proteins over the anterior sperm head (Bou Khalil et al. 2006). The sperm membrane also becomes hyperpolarized as potassium ions leave the sperm (Schreiber et al. 1998). Immediately after ejaculation, due to higher concentration of bicarbonate ions ( $\text{HCO}_3^-$ ) in the semen and female tract fluids,  $\text{HCO}_3^-$  is transported into sperm where, together with the  $\text{Ca}^{2+}$  ions, it activates sAC which later leads to initiating of cAMP-dependent PKA tyrosine phosphorylation by protein tyrosine kinase (PTK; Visconti et al. 1995). Thanks to that, spermatozoa become activated showing the low-amplitude, high-frequency flagellar beating with symmetrical waveforms (Yanagimachi 1970). Both sAC and PKA were shown to be essential for male fertility (Nolan et al. 2004; Hess et al. 2005). Moreover,  $\text{Ca}^{2+}$  ions activate phospholipase C (PLC; Finkelnstein et al. 2010) producing inositol triphosphate (IP3) and diacylglycerol (DAG) which in turn, via activation of phosphokinase C (PKC; Li et al. 2000), causes the hydrolysis of phosphatidylcholine by phospholipase D (PLD; Kam & Exton 2001) resulting in F-actin polymerization (Cohen et al. 2004).

Once the oocyte is released during the ovulation, spermatozoa change their motility pattern into hyperactivation characterized by asymmetrical flagellar beating with lower frequency and higher amplitude leading to more vigorous movement (Yanagimachi 1994). Hyperactivation of sperm motility is triggered by increase of cytoplasm  $\text{Ca}^{2+}$  levels (Suarez et al. 1987). Calcium ions can enter the sperm through membrane calcium channels such as essential CatSper channels (Qi et al. 2007) or can be released from intracellular stores, for instance the redundant nuclear envelope in the base of flagellum (Ho & Suarez 2001). Importantly, when  $\text{Ca}^{2+}$  level is raised too much, sperm become immotile indicating the calcium concentration-dependent motility response (Williams & Ford 2001). Mechanisms leading to an increase of intracellular  $[\text{Ca}^{2+}]$  are described in the next chapter. Hyperactivation apparently causes the sperm detachment from the binding with oviduct epithelium, sperm transport to ampulla (Demott & Suarez 1992) and it is also essential for passing through cumulus cells and for penetration of zona pellucida (Strauss et al. 1995).

As spermatozoa become capacitated and hyperactivated little by little, they are also gradually released which in turn prolongs the period when fertilizable spermatozoa are available in the oviduct (Eisenbach & Giojalas 2006). On the other hand, this continuous replacement is also important for preventing the polyspermy (Hunter & Leglise 1971). Besides already mentioned mechanisms, reactive oxygen species (ROS) are also important for the correct sperm function, including capacitation and hyperpolarization (De Lamirande & Gagnon 1993).

After releasing from the follicle, the oocyte-cumulus complex is carried to the fertilization site by the current of oviductal fluid induced by beating of ciliated epithelial cells. Ciliary beating seems to be in response to progesterone secreted by OCC as the progesterone receptor is localized in the lower half of ciliated oviduct epithelium (Teilmann et al. 2006). In addition, spontaneous contractions of smooth muscle of the oviduct are for the oocyte transport necessary as well. The spermatozoa possess the ability to, at first, percept this fluid flow and move against it. This orientated movement is called positive rheotaxis. What is more, the oviductal flow is also induced by coitus and lowers viscosity and clears the oviduct of debris (Miki & Clapham 2014). Prolactin released in response to sexual stimulation (Heiman et al. 1991) affects active secretion of  $\text{HCO}_3^-$  and water from the oviductal epithelium (Chen et al. 2010; Skowronski et al. 2011). Fluid viscosity in the female genital tract is the highest in the cervical mucous and in the isthmus and positively correlates with the flagellar propulsion – hyperactivated motility enables sperm to propel themselves through the viscous media (Suarez & Dai 1992).

Taken together, oviductal flow does not only select and guide capacitated sperm towards eggs but also, as it drags eggs down the oviduct, ensures that the fertilization happens in the oviduct and the fertilized egg and, therefore, has time to develop before its implantation. Rheotaxis seems to be essential for long-distance sperm guidance through the female reproductive tract (Miki & Clapham 2014).

Furthermore, as it can be observed in experiments *in vitro*, mammalian capacitated spermatozoa also sense the temperature gradient (Bahat et al. 2003). Even though the temperature difference between the warmer ampulla and cooler isthmus in response to ovulation was reported (Bahat et al. 2005), the distance between the fertilization and storage site seems to be too large for giving thermotaxis the essential role in sperm guidance. Moreover, the chemotactic responsiveness of spermatozoa correlates with capacitation as well (Cohen-Dayag et al. 1995).

#### 4. MOLECULAR CHARACTERIZATION OF CHEMICAL COMMUNICATION BETWEEN MAMMALIAN GAMETES

In this chapter, the most important known chemoattractants and signalling pathways which are involved in sperm guidance towards the egg in mammalian species are characterized. As research goes by, our knowledge and newly acquired evidence are constantly growing.

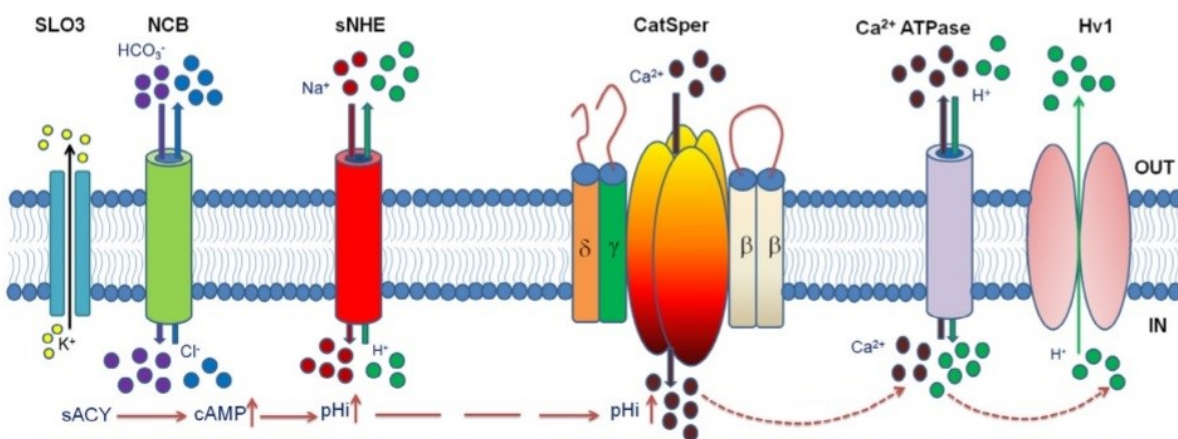
##### 4.1. *The calcium channel CatSper*

Calcium signalling is a key event for mammalian fertilization and many different calcium channels have been identified over the entire surface of sperm (see review Publicover et al. 2007). A remarkable breakthrough was achieved by the discovery of the cation channel of sperm (CatSper), a sperm-specific ion channel which is localized in the principal piece of sperm flagellum (Ren et al. 2001). As they control the calcium influx into sperm, CatSper channels are essential for hyperactivation (Qi et al. 2007), initiating sperm detachment from the isthmus, reaching the fertilization site (Demott & Suarez 1992) and penetration through zona pellucida surrounding the egg (Strauss et al. 1995).

The CatSper is a complex transmembrane heterotetramer formed by four  $\alpha$  subunits forming a pore (CatSper 1-4) and three auxiliary subunits CatSper  $\beta$ , CatSper  $\gamma$  and CatSper  $\delta$  (Ren et al. 2001; Liu et al. 2007; Wang et al. 2009; Chung et al. 2011). The presence of voltage sensors, positively charged residues of amino acids lysine and arginine, and abundance of histidine in  $\alpha$  subunits makes CatSper voltage-dependent and pH-sensitive, respectively (Ren et al. 2001). CatSper channel serves as a polymodal receptor for many diverse ligands associated with

fertilization – it binds progesterone, prostaglandins (Strünker et al. 2011; Lishko et al. 2011) and odorants (Brenker et al. 2012). Interestingly, these ligands activate CatSper directly without using the metabotropic pathways including cAMP or cGMP (Strünker et al. 2001). Remarkably, earlier studies informed about extracellular activation of CatSper by membrane-permeable cyclic nucleotide analogues, namely 8-Br-cAMP and 8-Br-cGMP (Kobori et al. 2000). Noteworthy is that there are several differences among mammalian species – for instance, murine CatSper is sensitive neither to progesterone nor to cyclic nucleotide analogues as it is observed in the case of humans (Lishko et al. 2011).

The CatSper channel cooperates with other channels and pumps of flagellar membrane (see Fig. 3). At first, its activation by increase in intracellular pH is mediated via  $\text{HCO}_3^-$  influx through  $\text{Cl}^-/\text{HCO}_3^-$  transporter (Chavez et al. 2012). Bicarbonate elevates the cAMP levels by activating sAC (Xie et al. 2006) which leads to opening of sperm-specific voltage-dependent  $\text{Na}^+/\text{H}^+$  exchanger (sNHE) and alkalisation of flagellar cytoplasm (Wang et al. 2003). Secondly, the change of membrane potential is caused by  $\text{K}^+$  efflux through sperm-specific pH-sensitive potassium channel KSper/SLO3 resulting in membrane hyperpolarization (Schreiber et al. 1998; Navarro et al. 2007). The level of intracellular  $\text{Ca}^{2+}$  ions, same like in all cells, is regulated by  $\text{Ca}^{2+}$ -ATPase (type IV) and by  $\text{Na}^+/\text{Ca}^{2+}$  exchanger which translocate  $\text{Ca}^{2+}$  out and protons with sodium into the flagellum, respectively (see review Blaustein & Lederer 1999; Schuh et al. 2004). The following acidification is controlled by proton extrusion via voltage-gated channel  $\text{H}^+$  (Hv1) (Lishko et al. 2010).



**Figure 3:** The regulation of CatSper channel accompanied by other channels and pumps of flagellum membrane: increase in intracellular pH caused by cAMP-dependent activation of sNHE exchanger together with the membrane hyperpolarization resulting from  $\text{K}^+$  efflux through KSper/SLO3 channel leads to entry of  $\text{Ca}^{2+}$  via CatSper channel.  $\text{Cl}^-/\text{HCO}_3^-$  transporter,  $\text{Ca}^{2+}$  ATPase and Hv1 channel are also involved in CatSper functioning. (Taken and modified from Singh & Rajender 2015).

The increase in intracellular  $\text{Ca}^{2+}$  concentration is propagated from the principal piece, where it starts, through the midpiece to the head of sperm (Xia et al. 2007). Calcium then binds to calmodulin and activates the calmodulin-dependent protein kinase (CaMK) that phosphorylates radial spoke proteins of the axoneme (Si & Olds-Clarke 2000). Calcium also activates cAMP-dependent tyrosine phosphorylation (Visconti et al. 1995) which was observed to be suppressed by higher  $[\text{Ca}^{2+}]_i$  as a response to the lack of ATP (Baker et al. 2004). The explanation for this contemplation was provided by recent study. In higher  $[\text{Ca}^{2+}]_i$  (4 mM) the activity of glyceraldehyde-triphosphate dehydrogenase (GADPH), a key enzyme of glycolysis that in sperm primarily generates ATP, is inhibited which leads to insufficient production of ATP. On the contrary, lesser  $[\text{Ca}^{2+}]_i$  (0,5-1 mM) stimulates the GADPH activity (Li et al. 2016).  $\text{Ca}^{2+}$  ions therefore have a biphasic role on sperm motility (via protein phosphorylation) and also on sperm metabolism.

However, not all ligands of CatSper channels stimulate the calcium entry. CRISP1 protein, a member of the mammalian cysteine-rich secretory protein family, was shown to be expressed by the cumulus cells and to inhibit the CatSper channels in capacitated sperm which, in turn, decreases the hyperactivated motility and leads to the linear movement straight toward the egg (Ernesto et al. 2015). All these findings put CatSper channels in a role of suitable non-hormonal targets for both male and female contraceptives.

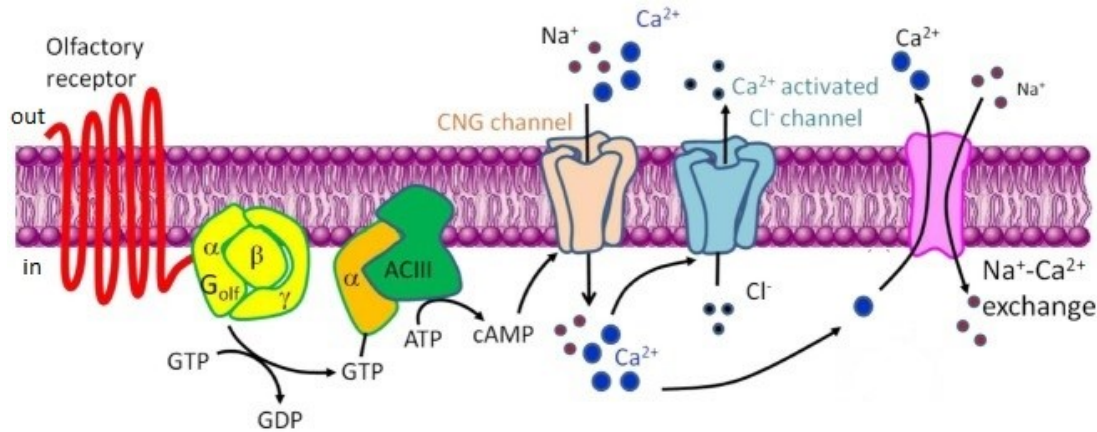
## *4.2. Compounds of odour perception*

### *4.2.1. Signal transduction in olfactory epithelium*

Many signalling mechanisms can be surprisingly found out of their primary functional tissues. Thus, along the female and male reproductive tracts compounds are often detected which are originally found somewhere else. Compounds of odour perceptions are the first example. Olfactory epithelium serves as one of the chemosensory systems in mammals. Volatile chemical compounds are detected in the nasal cavity by odorant receptors (ORs) expressed in cilia of the olfactory sensory neurons which directly lead to the brain (Shepherd 1988). ORs have seven transmembrane domains and belong to the super family of G protein-coupled receptors (GPCRs) (Buck & Axel 1991). OR genes include around 900 genes and pseudogenes clustered on all human chromosomes except 20 and Y and more than 1500 genes in the mouse genome (Glusman et al. 2001; Zhang & Firestein 2002). Odorant transduction pathway is activated after the binding of ligand to ORs (see Fig. 4). The G protein  $\alpha$ -subunit ( $G_{\text{olf}} \alpha$ ) dissociates from heterotrimeric G protein (Jones & Reed 1989) and activates the membrane form of adenylate cyclase III (ACIII) (Lowe et al. 1989). The elevation of cAMP



leads to symport of calcium and sodium ions via the cyclic nucleotide-gated channel (Nakamura & Gold 1987) and the  $\text{Ca}^{2+}$ -activated chloride channel triggers depolarization of membrane (Billig et al. 2011). Finally, the  $[\text{Ca}^{2+}]_i$  is controlled by  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (Pyrski et al. 2007) and plasma membrane  $\text{Ca}^{2+}$ -ATPase (Saidu et al. 2009).



**Figure 4:** Signal transduction in sensory neurons of olfactory epithelium: Once the OR is activated by specific odorant, a G protein ( $G_{olf}$ )-coupled cascade leads to synthesis of cAMP by ACIII.  $\text{Ca}^{2+}$  ions enter the sensory neuron via CNG channel and result in membrane depolarization as  $\text{Cl}^-$  ions leave the neuron through  $\text{Ca}^{2+}$ -activated chloride channel. One of the mechanism regulating the  $[\text{Ca}^{2+}]_i$  is the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger. (Taken from Kang & Koo 2012).

Interestingly, ORs are expressed also in non-chemosensory tissues – for example in the spleen and in the insulin-secreting  $\beta$  cells (Blache et al. 1998), erythroid cells (Feingold et al. 1999), myocardial cells (Drutel et al. 1995), non-olfactory neurons of the autonomic nervous system and the cerebral cortex (Weber et al. 2002; Otaki et al. 2004). Moreover, OR genes were also found in mammalian cells of the male germ line (Parmentier et al. 1992) and ORs in the midpiece of the flagellum of mature spermatozoa (Vanderhaeghen et al. 1993), suggesting their possible role in sperm chemotaxis. In addition, components of the olfactory signal transduction, such as ACIII,  $G_{olf}$  and CNG channels, were localized within the testis and male germ cells leading to the idea that testicular ORs use the same signalling pathway as those in the olfactory epithelium (Weyand et al. 1994; Defer et al. 1998).

#### 4.2.2. Odorant receptors and the mouse reproductive tract

The most well-known mouse olfactory receptor MOR23 (encoded by the *Olf16* gene) is expressed besides the olfactory epithelium also in the testis (Asai et al. 1996) and responses to the floral odorant lylal which is structurally an aldehyde (Touhara et al. 1999). Later study (Fukuda et al. 2004) specified the expression of MOR23 in spermatogenic cells, concretely into the round spermatids of approximately 30% of the seminiferous tubules. Gradient of lylal induces a dose-dependent increase of  $[\text{Ca}^{2+}]_i$  in both spermatogenic cells and in sperm. Calcium ions then, in turn, stimulate sperm chemotactic motility via asymmetric flagellar

beating (Fukuda et al. 2004). Intriguingly, MOR23 was also shown to regulate muscle cells migration and adhesion supporting the muscle regeneration (Griffin et al. 2009). Another mouse olfactory receptor, which is also expressed in testis, is MOR17 preferentially binding heptanal (Krautwurst et al. 1998).

In the mouse testis, except olfactory receptors, the pheromone receptors were identified as well (Tatsura et al. 2001). Pheromone receptors are originally expressed by sensory neurons of the vomeronasal organ, another peripheral chemosensory tissue majorly detecting pheromones in many vertebrate species (Wysocki 1985). Diverse pheromones are known for their possibility to affect the fear response to the presence of predator (Kobayakawa et al. 2007) same like the reproductive physiology and behaviour (Novotny 2003). Pheromone receptors can be divided into two groups, V1Rs and V2Rs, according to their expression in the apical or basal zone of the vomeronasal sensory epithelium, respectively (Ryba & Tirindelli 1997). Testicular vomeronasal receptors (TVRs) all belong to the V1R subfamily and were seen to be selectively expressed only by small subsets of spermatids in the late stage of the spermatogenesis, concretely by round and elongated spermatids (Tatsura et al. 2001). TVRs are similarly to the pheromone receptors expressed in the vomeronasal organ but not in the olfactory epithelium (Tatsura et al. 2001).

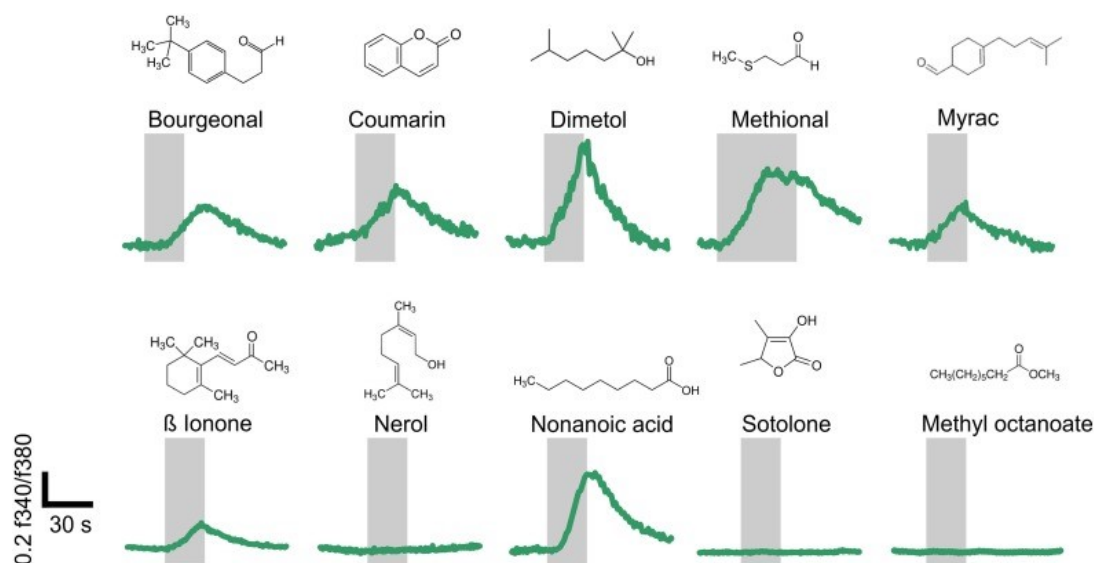
However, as it is complicated to identify the particular odorant or pheromone for these receptors found in both spermatogenic cells and sperm and even more complicated for the endogenous origin, we still cannot surely confirm their putative role in sperm chemotaxis *in vivo*.

#### 4.2.3. Odorant receptors and the human reproductive tract

Most of the ectopically expressed ORs among 15 inspected human tissues were found in the human testis (Flegel et al. 2013). Up to date, the identification of around 10 receptor-ligand pairs has been successful. For instance, the OR1D2 (hOR 17-4) is known for binding an odorant bourgeonal, which is responsible for the scent of lily of the valley (Spehr et al. 2003). Other pairs are OR7A5-myrac, OR4D1-PI-23472 (Veitinger et al. 2011), OR1C1/OR2J2-coumarin, OR8D1-sotolone (Adipietro et al. 2012), OR51E2- $\beta$  ionone (Neuhaus et al. 2009), OR51E1-nonanoic acid (Saito et al. 2009), OR52D1-methyl octanoate (Sanz et al. 2005), OR2W3-nerol, OR10J1-dimetol, OR2H1-methional (Flegel et al. 2015). It is noteworthy that besides these synthetic ligands, natural odorants were found in the female follicular fluid, concretely 5 $\alpha$ -androst-16-en-3-one for OR4D1, 4-hydroxy-2,5-dimethyl-3(2H)-furanone for OR7A5 and methional (Hartmann et al. 2013).

The recent study revealed the comprehensive assay about the compartment-specific placement of diverse ORs in human sperm which may indicate their specific function (Flegel et al. 2015). For example, the OR51E1 and OR51E2 were detected on the acrosome cap, OR6B2 in the equatorial segment (Flegel et al. 2015), OR1D2 in the midpiece (Neuhaus et al. 2006) and OR10J1 in the tail (Flegel et al. 2015).

The most common believe is that ORs on spermatozoa play a role in sperm chemotaxis via increasing the  $[Ca^{2+}]_i$  as it was reported for mice MOR23 (Fukuda et al. 2004) and human OR1D2 (Spehr et al. 2003). However, not all tested odorants mentioned above caused the elevation of  $[Ca^{2+}]_i$ , namely sotolone, methyl octanoate and nerol (see Fig. 5). Interestingly, the first two were identified as only antisense transcripts (Flegel et al. 2015). Moreover, with the discovery that odorants bind directly to CatSper channels and thus heighten the  $[Ca^{2+}]_i$ , without involving metabotropic pathways (Brenker et al. 2012), the role of sperm ORs in the sperm chemotaxis remains unclear and ORs more likely have a bigger importance in a different function.



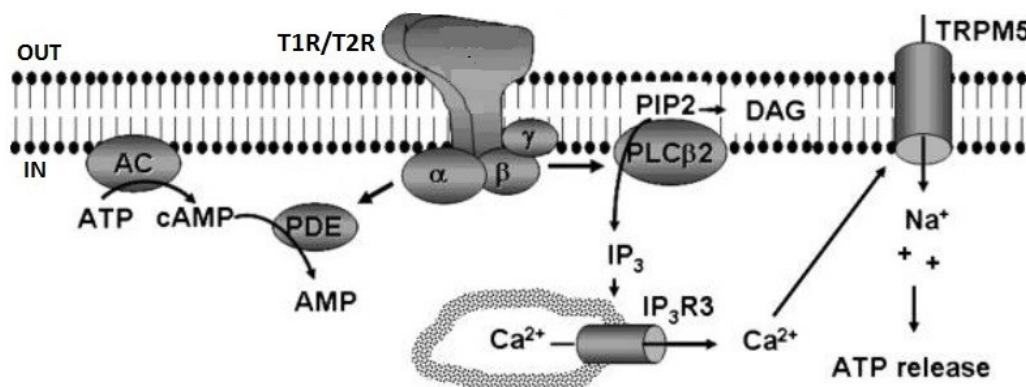
**Figure 5:** Calcium signals in human spermatozoa in response to stimulation of ORs by diverse odorants: Three of ten tested odorants (namely nerol, sotolone and methyl octanoate) did not evoke the elevation of  $[Ca^{2+}]_i$ . ORs were stimulated for 20-40 seconds (as indicated by grey bars). The  $[Ca^{2+}]_i$  was rated by a function of time of fluorescence ratio. (Taken from Flegel et al. 2015).

### 4.3. Compounds of taste perception

#### 4.3.1. Signal transduction in taste bud cells

Another example of unusual compounds found in genital tracts are those of the taste perception. Taste buds made up by modified epithelial taste cells serve as another mammalian chemosensory system. Taste receptors from the family of GPCRs are divided into two

subfamilies: the T1Rs (also called Tas1Rs) and T2Rs (Tas2Rs). T1Rs consist of three members which form heterodimers – T1R1 + T1R3 is responsible for the detection of umami compounds (monosodium glutamate and l-amino acids) on the one hand (Nelson et al. 2002) and T1R2 + T1R3 for the detection of sweet taste on the other hand (Nelson et al. 2001). In addition, T2Rs are known as receptors for bitter taste (Chandrashekar et al. 2000). On the contrary, the perception of sour and salty tastes occurs via ion channels (Huang et al. 2006). T1Rs and T2Rs share the similar signalling pathway (Zhang et al. 2003; see Fig. 6). As they are associated with heterotrimeric G protein which is composed of  $\alpha_{\text{gust}}$  (gustducin, Gnat3), G $\beta$ 3 and G $\gamma$ 13 subunits (Wong et al. 1996), the tastant-binding to its receptor activates the phospholipase C type  $\beta$ 2 (PLC $\beta$ 2) (Rössler et al. 1998), which produces inositol triphosphate and diacylglycerol, both known as second messengers. IP $_3$  binding to its receptor type III (IP $_3$ R3) on the intracellular calcium store (Miyoshi et al. 2001) leads to the releasing of Ca $^{2+}$  ions which open the transient receptor potential channel (TRPM5) through which Na $^{+}$  ions enter the taste cell. The membrane depolarization facilitates the releasing of ATP (Perez et al. 2002). T1Rs also regulates the intracellular levels of cAMP by activation of the phosphodiesterase (Nakagawa et al. 2009).



**Figure 6:** Signalling pathway in taste cells: After ligand-binding to the taste receptor (T1R/T2R),  $\alpha$  subunit of trimeric G protein activates PLC $\beta$ 2 which produces IP $_3$  and DAG. As IP $_3$  binds to its receptor IP $_3$ R3, released Ca $^{2+}$  ions open TRPM5 channel and Na $^{+}$  influx-caused depolarization releases ATP.  $\alpha$  subunit also lows levels of cAMP by activation of PDE. (Taken and modified from Kinnamon 2009).

Similarly to the olfactory receptors, taste receptors can also be found out of their primary tissue. T2Rs are alone expressed for instance in the respiratory system, concretely in the nasal cavity and lungs (Finger et al. 2003; Shah et al. 2009) and together with the T1Rs in the digestive system (Rozengurt et al. 2006; Margolskee et al. 2007), in the central nervous system (Ren et al. 2009; Singh et al. 2011) and not of minor importance also in the testis (Li & Zhou 2012; Mosinger et al. 2013).

#### 4.3.2. Taste receptors type I (T1Rs) in the male genital tract

T1R3 together with  $\alpha_{\text{gust}}$  were observed to be expressed in the testis, specifically in the elongating and already elongated spermatids (Mosinger et al. 2013). The study with mouse testis revealed the different expression pattern of T1R3 and  $\alpha_{\text{gust}}$  not only during spermatogenesis but also during the mouse development (Gong et al. 2016). In testis of prepubertal mice, only  $\alpha_{\text{gust}}$  was detected in apoptotic spermatogonia. At onset of puberty, both proteins were highly expressed in late spermatids, whereas the decreased expression was observed in aged mice. Their putative role in the sperm development was confirmed with the discovery that male mice lacking testicular T1R3 and  $\alpha_{\text{gust}}$  genes are infertile as a likely result of the disruption of signalling pathways that involve these missing compounds (Mosinger et al. 2013). Similarly, the diverse pattern was found also in Leydig cells with the highest expression of both T1R3 and  $\alpha_{\text{gust}}$  after the puberty (Gong et al. 2016). T1R2 was detected in mice in primary spermatocytes and spermatogonial cells (Iwatsuki et al. 2010). In addition, TRPM5 was detected only in the later spermatids and the gamma subunit 13 (G $\gamma$ 13) is expressed during spermatogenesis, too (Li & Zhou 2012).

T1Rs and their signalling compounds are localized in the released spermatozoa of many mammalian species as well. T1R1 and T1R3 of the umami receptor were observed to be expressed in the flagellar principal piece and on the acrosomal cap (Meyer et al. 2012).  $\alpha_{\text{gust}}$  has the same localization and, moreover, can also be found in the midpiece (Fehr et al. 2007). Interestingly, the T1R1, likely together with T1R3, has an essential role in suppressing the sperm maturation processes during the sperm journey through the female genital tract until the reaching of the vicinity of the egg (Meyer et al. 2012). The concentration of glutamate (the ligand for umami receptor; Nelson et al. 2002) is the highest in the uterus and is descending in the direction to the ampulla of the oviduct (Harris et al. 2005).  $\alpha_{\text{gust}}$  thus dissociates from the G protein and activates the phosphodiesterase which lowers the cAMP levels. cAMP hydrolysis is later diminished by decreasing the receptor activity and the sperm maturation is triggered by the increase of the  $[\text{Ca}^{2+}]_i$ , same like the activity of sAC in response to higher  $\text{HCO}_3^-$  levels (Meyer et al. 2012). The importance of T1R1 in regulating the spontaneous acrosome reaction was confirmed by experiments with *T1R1*-null mice (Meyer et al. 2012).

#### 4.3.3. Taste receptors type II (T2Rs) in the male genital tract

Taste receptors from the subfamily of T2Rs are also expressed in male germ cells. Both human and mice T2Rs are localized in seminiferous tubules (Xu et al. 2013). Different studies revealed their expression to spermatocytes going through meiosis and to post-meiotic round

spermatids (Li & Zhou 2012; Xu et al. 2013). The localization of bitter receptors is altered after the maturation of testicular spermatids to epididymal sperm – the proteins of sperm cell, including T2Rs and their signalling components, are redistributed during this maturation resulting in their higher concentration in the cytosol-rich midpiece and the acrosome (Xu et al. 2013). T2Rs were found to be important for regulating the spermatogenic cycle as the *T2R*-mutant mice have smaller testis and in most of the seminiferous tubules the spermatid phase is ablated (Li & Zhou 2012).

In conclusion, taste receptors and components of their signalling pathways expressed in male germ cells may have several roles: firstly, they are likely to regulate the spermatogenesis (Li & Zhou 2012; Mosinger et al. 2013), secondly, they detect diverse chemical substances of their surrounding environment, especially the toxic and potentially harmful chemicals in the lumen of seminiferous tubules same like in the female genital tract (Li & Zhou 2012), thirdly, as they are characterized by the distinct segmental-distribution and increasing the calcium levels in sperm, they might be involved also in regulating the sperm chemotaxis or sperm motility (Fehr et al. 2007 ; Meyer et al. 2012). Finally, taste receptors also appear to play a part in other cellular and molecular processes which involve the calcium signalization such as the formation of flagella and acrosome, histone modifications or remodelling and packaging of chromatin (Xu et al. 2013).

#### *4.4. Formyl peptide receptors*

Formyl peptide receptors (FPRs), other members from the family of GPCRs with seven transmembrane domains (Prossnitz & Ye 1997), are originally found on phagocytic leukocytes, namely on neutrophils and/or monocytes which are by diverse mechanisms responsible for inflammation and protection of an organism from pathogens (Durstin et al. 1994). Up to date, three human (Bao et al. 1992) and eight mouse (Gao et al. 1998; Wang & Ye 2002) members of FRP family have been identified. Their various ligands, N-formylated oligopeptides derived from degradation of bacterial (Marasco et al. 1984) or mitochondrial proteins (Carp 1982), serum amyloid A (Su et al. 1997),  $\beta$ -amyloid peptide (Le et al. 2001) or lipid lipoxin A4 (Takano et al. 1997) serve as leukocytes chemoattractants. The first described and therefore also the well-known ligand is the N-formyl-methionyl-leucyl-phenylalanine (fMLP; Showell et al. 1976).

Different FPRs are associated with different G proteins, however, they initiate the same signalling pathways. First, the dissociated subunits of G protein activate phosphoinositide 3-kinase (PI3K; Pan et al. 2000) and phospholipase C (Camps et al. 1992) hydrolysing the

membrane phospholipid into inositol triphosphate and diacylglycerol. IP<sub>3</sub> triggers the calcium release from endoplasmic reticulum and DAG activates protein kinase C (Li et al. 2000). Second, the small regulatory GTPase RAS through interaction with protein kinase RAF activates the mitogen-activated protein kinases (MAPK) cascade (Rane et al. 1997) leading to the production of reactive oxygen species (Paruch et al. 2005). Here, it is important to note that both pathways result in activation of protein tyrosine kinase as it can be observed during the process of sperm capacitation prior to acrosome reaction. Finally, the membrane ADP-ribosyl cyclase CD38 converts NAD<sup>+</sup> into cyclic ADP-ribose (cADPR; Howard et al. 1993) which in turn elevates the [Ca<sup>2+</sup>]<sub>i</sub> by either binding to ryanodine receptor-gated calcium stores or by causing the influx of Ca<sup>2+</sup> ions from extracellular side (Partida-Sanchez et al. 2001).

It is important to note that FPRs induce chemotactic movement not only in leukocytes (Showell et al. 1976) but also in sperm (Vijayasathya et al. 1980). FPRs expressed in flagellum membrane cause via increase of [Ca<sup>2+</sup>]<sub>i</sub>, hyperactivated sperm motility and likely also the acrosome reaction indicating their diverse function (Gnessi et al. 1986). What is more, some members of rodent FPRs family were identified in testis (Wang & Ye 2002) and surprisingly seen to be selectively expressed by small subsets of neurons dispersed in the vomeronasal organ (Liberles et al. 2009) where they have been reported besides two types of pheromone receptors (Ryba & Tirindelli 1997) as a third group of chemosensory receptors (Liberles et al. 2009). The growing evidence points at the fact that FPRs expression can be also observed in the cells of central nervous system (Sozzani et al. 1995; Becker et al. 1998), concretely in neural stem cells where FPRs play a pivotal part in proliferation, migration and differentiation (Wang et al. 2016).

#### *4.5. Hormones*

##### *4.5.1. Progesterone*

Progesterone, one of the steroid hormones synthesized from cholesterol, is a key regulator mediating various events connected with reproduction. In the female genital tract, it is secreted by granulosa cells within the follicle of ovaries (Park & Mayo 1991). After ovulation, the secretion is ensured by cumulus cells surrounding the oocyte (Vanderhyden & Macdonald 1998) and forms the concentration gradient along the cumulus cell mass (Teves et al. 2006). Progesterone, therefore, can be found in both follicular and oviductal fluids. In general, as steroid hormones are lipophile, they pass through the cytoplasmic membrane and after binding to their cytoplasmic receptors, they are translocated to the nucleus where they as transcription factors regulate the gene transcription (Evans 1988). An intriguing role of

progesterone was revealed by observation that mammalian sperm exposed to concentration gradient of progesterone exhibit the chemotactic response (Villanueva-Diaz et al. 1995). Thus, progesterone was postulated to be a sperm chemoattractant active at the picomolar range (Teves et al. 2006).

Interestingly, two nongenomic progesterone receptors have been found in the sperm cell membrane (Blackmore et al. 1991; Luconi et al. 1991). It was believed that progesterone stimulates tmAC synthesising cAMP which, in turn, by activation of PKA leads to tyrosine phosphorylation (Teves et al. 2009) and that progesterone elevates the  $[Ca^{2+}]_i$  in sperm, as already observed decades ago (Blackmore et al. 1990), by calcium mobilization from intracellular stores (Bedu-Addo et al. 2008). However, it was lately reported that progesterone in humans induces a rapid calcium influx via sperm-specific calcium CatSper channels (Lishko et al. 2011; Strünker et al. 2011) which are localized in the flagellar principal piece (Ren et al. 2001). Notably, murine CatSper channels are known to be insensitive to progesterone (Lisho et al. 2011).

Besides chemotactic activity (Teves et al. 2006), progesterone was shown to possess the ability to stimulate, mainly via CatSper channels, hyperactivation of sperm motility (Gwathmey et al. 2000; Strünker et al. 2011) and to induce (likely together with zona pellucida) the acrosome reaction (Osman et al. 1989; Roldan et al. 1994). Moreover, progesterone receptors are also expressed along the female reproductive tract, for instance in the lower part of motile cilia of the oviductal epithelium (Teilmann et al. 2006) where upon stimulation by progesterone secreted by passing oocyte-cumulus complex enhance the ciliary beating and thus facilitate the OCC transport to the fertilization place (Wessel et al. 2004).

#### 4.5.2. *Natriuretic peptides (ANP, CNP)*

The family of natriuretic peptides consists of three hormones – atrial natriuretic peptide (ANP; de Bold et al. 1981), B-type (brain) natriuretic peptide (BNP; Sudoh et al. 1988) and C-type natriuretic peptide (CNP; Sudoh et al. 1990). All natriuretic peptides undergo two cleavages during the synthesis of their active form – following the gene transcription, precursors preprohormones are cleaved into prohormones which are stored in secreting cells. Upon release, they are cleaved again into an active form (Oikinawa et al. 1984; Tawaragi et al. 1991; Ogawa et al. 1994).

Concretely, proANP is mainly stored in the cardiac atrial granules (Oikinawa et al. 1984) and after releasing it is converted by corin, a cardiac serine protease (Yan et al. 2000). ANP is known for its essential role in modulating the homeostatic blood pressure (de Bold et al.



1981) together with suppressing the cardiac hypertrophy and fibrosis (Itoh et al. 1990). Interestingly, the presence of ANP in the female reproductive tract was reported, namely in ovaries (Kim et al. 1992), follicular fluid (Anderson et al. 1994) and even in oocytes (Kim et al. 1993). Furthermore, the expression of A-type natriuretic peptide receptor (NPRA), the common receptor for both ANP and BNP (Chinkers et al. 1989), was observed in human spermatozoa indicating its possible role in fertilization (Silvestroni et al. 1992). The NPRA forms a transmembrane homodimer with extracellular binding site and guanylyl cyclase linked to the intracellular domain (Chinkers et al. 1989). The receptor activity is controlled by phosphorylation, as the dephosphorylation leads to receptor desensitization (Potter & Garbers 1992). ANP has two positive effects on spermatozoa: first, it induces the chemotactic response of sperm (Zamir et al. 1993) and second, it triggers the acrosome reaction likely by elevating the intracellular levels of cGMP (Anderson et al. 1994). However, it remains unconfirmed whether ANP functions as a sperm chemoattractant also *in vivo* due to the presence of neutral endopeptidase, the inhibitor of ANP (Vanneste et al. 1988), in the seminal fluid (Zamir et al. 1993).

In addition, CNP together with its B-type natriuretic peptide receptor (NPRB) intracellularly synthesising cGMP as well (Chang et al. 1989) were found to be expressed in the reproductive tract of both females and males. On the one hand, they are essential for functional development of murine ovaries and uterus (Tamura et al. 2004) and for preventing that oocytes do not meiotically mature untimely (Zhang et al. 2010). On the other hand, they were localized in the epididymis, prostate (Nielson et al. 2008) and testis where they regulate the microenvironment during spermatogenesis (Xia et al. 2007). CNP alone is present in the seminal fluid (Chrisman et al. 1993) but not in sperm (Xia et al. 2016). On the contrary, NPRB was detected in human spermatozoa, concretely in the acrosomal head region and the initial part of the tail, and it was shown to positively affect the sperm motility and to induce the acrosome reaction suggesting its role in sperm fertility (Xia et al. 2016).

#### 4.6. Cytokines (*chemokines, defensines*)

Chemokines belonging to the great family of cytokines are contained in the female as well as male genital tract fluids. The important member called RANTES (regulated on activation normal T-cells expressed and secreted) chemokine is secreted by granulosa cells into the follicular fluid (Machelon et al. 2000). The uterine fluid and seminal plasma comprises RANTES as well (Hornung et al. 1997; Naz & Leslie 2000). Remarkably, RANTES was seen to induce the chemotactic response not only in leukocytes, namely monocytes, T-lymphocytes

(Schall et al. 1990) and eosinophils (Alam et al. 1993) but also in human spermatozoa. Additionally, the spermatozoa express the CCR1 and CCR5, CC chemokine receptor type 1 and type 5 (Isobe et al. 2002), which function as receptors for RANTES (Gao et al. 1993). It was also observed that levels of RANTES are elevated during diseases influencing fertility, such as endometriosis (Khorram et al. 1993) and pelvic pain syndrome (Penna et al. 2007), leading to premature acrosome exocytosis triggered by cAMP increase and thus to loss of sperm fertilizing ability (Barbonetti et al. 2008).

Besides RANTES, other chemokines like monocyte chemotactic protein 1 (MCP-1) and macrophage inflammatory protein 3 $\alpha$  (MIP-3 $\alpha$ ) were also detected in follicular fluid (Kawano et al. 2001; Caballero-Campo et al. 2014). Moreover, female genital tract contains  $\beta$ -defensins responsible for protection against infection (Valore et al. 1998). Spermatozoa were seen to express in their flagellum the CCR6, CC chemokine receptor type 6 (Caballero-Campo et al. 2014), which serves as a common receptor for MCP-1, MIP-3 $\alpha$  and  $\beta$ -defensins (Greaves et al. 1997; Yang et al. 1999) and similarly modulates sperm motility and chemotaxis (Caballero-Campo et al. 2014). The possible importance of further chemokines in influencing the sperm motility cannot be excluded.

## 5. CONCLUSION

Chemical communication of gametes has become a field of interest of many scientists for several decades and since that time, great breakthroughs have been done. The main columns of our knowledge come from experiments with marine invertebrate species which often serve as model organisms and are therefore well-studied. Their spermatozoa respond to diverse species-specific egg-delivered peptides essential for triggering the progressive motility and for changes in motility pattern. On the contrary, a few statements about sperm chemotaxis in mammalian species are generally acknowledged as well, however, the current evidence is undoubtedly not complete. The oocyte-cumulus complex serves as a key origin of chemoattractants with progesterone as the most significant example. Their secretion is under the control of oestrous cycle and initiate processes like sperm capacitation, hyperactivation and acrosome reaction. Besides signalling pathways exclusively found only by either invertebrates or mammals, some of them, like those induced by CatSper channels, are shared indicating the possible evolutionary parallel. Generally, the biggest obstacle lies in the problematic mimicking of a unique environment within the female genital tract in experiments *in vitro*. In addition, many described sperm chemoattractants are synthetic and

their naturally occurring analogues are not identified yet. Thus, it is not surprising that we can expect further researches aimed at such a topic. The complex understanding of sperm guidance mechanism on molecular level brings novel and intriguing insights into many fields of science which are related to physiology and reproduction. Nowadays, new findings are essential especially for successful treatment of human infertility.

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