Canine seminal plasma proteins

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Abstract

Researchers have been done to identify a proteins contains in canine seminal plasma and relate them to quality, sperm motility, vigor and their role on capacitating, acrosome reaction, hyperactivation and etc. In addition, determination of specific canine seminal plasma proteins serving as biomarkers of some reproductive diseases. It is known that proteins, like: spermadhesin protein family, osteiopontin, kallikrein, some heparin-binding proteins and etc. are associated with sperm quality and play an important role during fertilization. Also, there is a data that specific proteins have an effect on hyperactiovation on canine spermatozoa, gamete interaction and etc. Specific seminal plasma proteins have been identified as markers for some diseases.

Thus the present literature review aims to append to more growing studies demonstrates that the canine seminal plasma proteins have a different impact and also to give new insight of aspects related to the wide roles of seminal plasma proteins and their effect and influence on fertilization process.

Key words: effects, seminal plasma proteins, dog

Семенни плазмени протеини при кучета

Деница Даскалова

Департамент по Репродуктивни биотехнологии и криобиология на гамети, Институт по биология и имунология на размножаването „Акад. Кирил Братанов" при БАН – София

Резюме

Извършени са редица изследвания за идентифициране на протеини, съдържащи се в семиналната плазма от куче, за установяване на тяхната роля в подвижността, кинетиката, в процеса на капацитацията, както и определянето на специфични протеини, служещи като биомаркери за някои репродуктивни заболявания. В допълнение, известно е, че определени семинално плазмени протеини като: семейството на спермадезините, остеопонтина, каликреина, някои от хепаринсвързващите протеини и др., са свързани с качеството на спермата и играят важна роля по време на процеса на опложданe. Установени са и протеини от семиналната плазма, оказващи ефект върху хиперактивацията на сперматозоиди от куче, взаимодействието между гаметите и др. Идентифицирани са семинално плазмени протеини, служещи, като маркери за редица репродуктивни болести.

Целта на настоящия обзор е да се добавят нови данни към все по-нарастващите проучвания, установяващи, че семинално плазмените протеини имат много и различни роли.

Ключови думи: ефект, семинално плазмени протеини, кучета

Introduction

The semen is a heterogeneous complex of a cell suspension in a protein-rich fluid (Rodríguez-Martínez et al., 2011; Aquino-Cortez et al., 2016). Secretions produced by the testicles, epididymis, and accessory sex glands constitute the seminal plasma (SP). The prostate is the only accessory sex gland of dogs, and its fluid constitutes approximately 95% of the ejaculate (Aquino-Cortez et al., 2016). According, Mogielnicka-Brzozowska et al. (2012) some canine seminal plasma proteins (SPP) have been identified and established that these proteins can affect quality and fertility of spermatozoa. Newly, it was found that specific SPP contains in SP could be used as biomolecular markers for the diagnosis of some disease (azoospermia, prostate pathologies such as benign prostatic hyperplasia disease (BPH) and obstruction of the vas deferens or epididymis) (Schafer-Somi et al., 2013; Pandapotan et al., 2020). In addition, there is data that some SPP have an important role of process of capacitation; interaction and fusion of the gametes, acrosome reaction and hyperactivation of the sperm cells (Daskalova et al., 2017 a; Ickowicz et al., 2012; Suarez, 2008; Burkman, 2020). Nevertheless, numerous questions remain for role and effects of SPP are still not fully understood.

Discussion

It is known, that some SPP protect the sperm by binding to the plasma membrane (PM) during ejaculation and play role in sperm maturation, capacitation, acrosome reaction, hiperactivation and sperm-egg fusion (Primakoff et al., 2002; Aitken et al., 2007). Members of the spermadhesin protein family, respectively: two sperm surface proteins: PSP-I and AWN-1 are isolated from SP and play an important role during fertilization (Manaskova et al., 2002; 2008; Teixeira et al., 2006). The spermadhesins are multifunctional proteins with molecular weight (MW) between 12-16 kDa, whose biological activities depend on their sequence, grade of glycosylation or aggregation state, as well as on their ability to bind heparin or not, as they attach in varying degree, to the PM from the testis to the ejaculate. The spermadhesine have been related to multiple effects on spermatozoa including PM stabilization, capacitation and interplay between sperm oviductal lining or sperm-zona pellucida. Some heparin bind proteins seem to stabilize the PM over the acrosome prior to capacitation (Rodriguez-Martinez et al., 2011). The proteins PSP-I/PSP-II heterodimer is a protein of SP that is able to preserve *in vitro*, the viability, motility and mitochondrial activity of spermatozoa in many species, including in dogs. A positive relationship between the quantity of PSPI/PSPII and sperm motility was also found also, in humans (Aquino-Cortez et al., 2016). Some positive effects exerted by canine spermadhesins could occur due to albumins. The protein PSP-I prevents premature capacitation and acrosome exocytosis. Whether, these proteins are also involved in the interaction between spermatozoa and oviductal epithelium during sperm capacitation remains to be explored (Mogielnicka-Brzozowska et al., 2012). A part of canine SPP binds to the PM of the spermatozoa during ejaculation and blocking the progesterone receptors located in the acrosome region. Coating proteins and glycoproteins of prostatic origin are gradually removed during capacitation and other SPP can bind to the exposed receptors to induce the acrosome reaction (Aquino-Cortez et al., 2016). In addition, big protein family thought to be involved in capacitation and modulation of the

acrosome reaction belongs to the heparin-binding proteins (HBP) group. After ejaculation, HBP binds to the spermatozoa PM and affect fertility due to their modulatory role during the acrosome reaction and subsequent capacitation (Henao, 2018). Recently, it is observed that SPP identified as HBP with MW 61.5 kDa, are involved to the acrosome reaction on canine spermatozoa (Souza, 2006). According Daskalova et al., (2017 a) there is data, that canine SPP with high MW provide better motility and kinetic Computer-Assisted Sperm Analysis (CASA) parameters of the spermatozoa during *in vitro* capacitation conditions. It was demonstrated, that certain canine SPP have an important influence on the hyperactivation of the spermatozoa and more specifically, SPP with MW between 65 kDa and 80 kD have a beneficial effect on hyperactivity of the canine spermarozoa. In this regard, these SPP with high MW have an influence on some CASA motion kinetics parameters connected with hyperactivation of spermatozoa and more precisely – curvilinear velocity (VCL, μm/s) straightness (STR, %), linearity (LIN, %) and amplitude of lateral head displacement (ALH, μm/s) (Tsvetkov et al., 2019, Cancel et al., 2000). Another protein, found in canine SP and in other species (bull, boars, equines, bovines) is osteopontin (OPN). OPN has been associated with successful fertilitization (Cancel et al., 1999; Pardede et al., 2020). Briefly, OPN was positively correlated with fertility in stallions (72 kDa and pI 5.6), bull sperm (55 kDa and pI 4.5) and pig (Hao et al., 2006). The OPN has been localized on ejaculated bull sperm and may play a role in fertilization and also as a block to polyspermy. In the pig, addition of OPN during *in vitro* fertilization also reduced polyspermy rates and improved embryo development after fertilization (Pandapotan et al., 2020). Earlier studies, also showed that the SPP of mammals play a significant role in several essential steps such as sperm motility, sperm functionality such as regulating capacitation and gamete interaction and fusion and individual proteins from the same family carry out their functions in a species-specific manner (Rodríguez-Martínez et al., 2011). The protein reported as seminal plasma phospholipase A2 (PLA2) is thought to be involved in sperm capacitation in dogs, bovine and humans. The proteins identified as zinc-binding proteins (ZnBP) could involve in recognition during the fertilization of the gametes. In canine SP was established that zinc-binding protein family contains proteins with MW between 11.6 kDa to 152.3 kDa and these molecules can affect sperm fertility (Mogielnicka-Brzozowska et al., 2012). Two proteins with MW of 58.6 kDa and 67 kDa were positively associated with motility, vigor, morphology and PM integrity of the normal canine spermatozotizoa (Souza et al., 2007). It is considered, the kallikrein group proteins and particularly arginine esterase is a protein with role on the regulation of canine sperm motility. In addition, according Levy et al. (2014) these esterase is used as a biomolecular marker for the diagnosis of canine with benign prostatic hyperplasia (BHP) (arginine esterase concentration is higher in the serum of dogs with BHP). Proteins identified as lactoferrin, with MW of 75.2 kDa has a strongly bind to the PM of the spermatozoa and an iron-binding protein. The lactoferrin showed a significant positive correlation with the canine sperm concentration (Kikuchi et al., 2003). However, SPP with MW of 23 kDa, identified as phosphatidylethanolamine-binding protein, binds to a receptor on the PM appears to involve a glycosylphosphatidylinositol anchor. It is located on the acrosomal cap, the post-acrosomal region and the flagellum of mouse, human and canine spermatozoa and is suggest, that this protein plays role in capacitation, as decapacitation factor (Gibbons et al., 2005). Interesting, some specific SP components have been described as decapacitation factor in boars, stallion, humans and other species and are assumed to protect sperm from factors in the female tract that could trigger early capacitation, thus reducing the possibility of sperm oocyte interaction (Töpfer et al., 1998). Canine SPP with lower MW of 9 to 54 kDa seems have decapacitation effects to spermatozoa (Tsvetkov et al., 2019). In addition, according Akaki et al. (2016) SPP with low MW 10 kDa could inhibit the initiation of the capacitation. Protein called seminal vesicle autoantigen (SVA) was described in

mouse males. This protein has the ability to form complexes with zinc ions and bind choline phospholipids of Sperm PM. SVA acts as a decapacitation factor (Huang et al., 2007). The role of SVA in canine semen has not been well studied. However, Mass Spectrometry (MS) based protein identification of SPP in canine SP, could provide a better understanding of reproductive physiology, improving of different preservation and storage medium of the spermatozoa and assisting in the development of new storage protocols of the sperm cells. Some seminal plasma proteins serve as marker for various diseases – cancer and benign prostatic hyperplasia. The first protein considered as such a marker in humans was prostate acidic phosphatase (AcP) and prostate specific antigen (PSA) (Taira et al., 2007). PSA shows high homology to the main protein of canine prostate secretions – canine secretory prostatic esterase (CPSE) (Aquino-Cortez et al., 2016). Both enzymes belong to serine proteinase group and show similar activity in relation to protein substrates. The canine secretory prostatic esterase is a trypsin-like enzyme and PSA shows chymotrypsin activity, they both possess similar MW of 29 kDa and 34 kDa. Lactose dehydrogenase C4 (LDH-C4), which occur in SP, originates mainly from damaged or destroyed cells and can be a molecular marker of seminiferous epithelium condition. Lipocalin-typeprostaglandin synthase can serve as a marker for azoospermia in humans. It is a glycoprotein of 26 kDa. Functions of this enzyme in the male reproductive system have not been fully clarified yet; how-ever, it is considered that it participates in sperm maturation in the epididymis (Dube et al., 1994). Low concentration of protein identified as Carnitine or alkaline phosphatase (AP) is observed in canine azoospermic ejaculate. Alkaline phosphatase (AP) in canine seminal plasma arises predominantly from the cauda epididymis, making it a useful epididymal marker when evaluating azoospermia (Johnston, 2003). The SPP have an ample panorama of role and their effects to fertility are though object of further research. However, it is proved that, SPP modulate crucial functions and events of reproduction, such as sperm motility and capacitation, cell protection, acrosome reaction, fertilization and embryonic development (Camargo et al., 2018).

With this review we supplement a new data to the knowledge on effects of canine proteins contains in SP. Dogs are the best experimental model for comparative studies with humans due to the similarity of their respective accessory sex glands and prostate growth (DeLazari et al., 2018). In addition, studies on canine SP components provide information that enables the understanding of SPP function. At all of these levels, detailed studies on the composition of canine SP and its specific biological function are still needed. Such molecules, in both animals and humans, are potential candidates for fertility markers as well as markers of pathophysiological processes (Szczykutowicz et al., 2019).

Conclusion

In conclusion, the SP serves not only as a medium to carry, protect and nourish sperm after ejaculation, but also as a modulator of sperm function. The present review was conducted to append more data to growing evidence demonstrates that the SPP apparently have an extensive range of action and function to spermatozoa.

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