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Molecular mechanisms involved in sperm-oocyte interaction and oocyte activation. Infertility associated with the dysfunction of these mechanisms.

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Resum

La infertilitat afecta entre un 10 i un 15% de la població mundial actual, i s'associa tant a desordres i alteracions del sistema reproductor femení com del masculí. D'altra banda, la infertilitat idiopàtica, que és aquella que no es pot atribuir ni al factor femení ni al masculí, afecta aproximadament a un 25% de les parelles infèrtils en edat de reproduir-se. És doncs en aquest context on es centra l'objectiu principal del treball que és conèixer els mecanismes moleculars involucrats en la interacció dels gàmetes. Aquest coneixement podria servir com a punt de partida per relacionar la disfunció d'aquests mecanismes amb la infertilitat.

Amb aquest objectiu, s'ha dut a terme una revisió sistemàtica de la literatura actual extreta de la plataforma PubMed. Aquesta revisió sistemàtica ha estat sotmesa a uns criteris de selecció i a una anàlisi posterior de la seva qualitat, amb la finalitat d'establir una sistemàtica d'inclusió/exclusió dels diferents articles. A partir dels articles seleccionats, s'ha estudiat el procés de fecundació en els mamífers. Aquest procés consisteix en una conjunt d'etapes complexes on diferents proteïnes, tant espermàtiques com oocitàries, desenvolupen rols essencials que garanteixen aquesta interacció gamètica. Així doncs, l'estudi de la funció i participació d'aquestes proteïnes és fonamental per tal comprendre i tractar la infertilitat idiopàtica.

Durant la reacció acrosòmica que pateix l'espermatozoide, que és un procés indispensable perquè aquest adquireixi la capacitat de fecundar l'oòcit, hi ha vàries proteïnes espermàtiques, sobretot la PKA i altres de la família de les SNAREs que tenen un paper clau. Endemés, els enzims hidrolítics alliberats després d'aquesta reacció acrosòmica, com l'acrosina, i els receptors de progesterona i de proteïnes de la ZP són imprescindibles perquè l'espermatozoide pugui penetrar la ZP. Posteriorment, es produeix la interacció i fusió de les membranes del gàmetes, esdeveniment en el qual la proteïna espermàtica IZUMO1 i la proteïna oocitària JUNO tenen una funció preeminent. En aquest sentit, s'ha observat que deficiències en ambdues proteïnes impossibilita que es produeixi la fusió dels gàmetes, la qual cosa s'associa amb problemes severs d'infertilitat. Finalment, després de la fusió de les membranes, es desencadenen una sèrie d'oscil·lacions de calci citosòlic que permeten l'activació de l'oòcit. En aquest procés, que és necessari perquè l'oòcit conclogui la segona divisió meiòtica, la proteïna específica espermàtica PLCζ hi juga un rol fonamental. Tanmateix, sembla que aquesta proteïna no duria a terme aquesta funció en solitari, sinó que n'hi ha d'altres com la PAWP que també hi podrien estar involucrades.

En definitiva, es fa molt necessari ampliar el coneixement de les proteïnes espermàtiques i oocitàries que participen en els esdeveniments que es produeixen abans, durant i després de la fecundació per tal de comprendre de quina manera la disfunció dels diferents mecanismes moleculars involucrats en aquests processos estan relacionats amb la infertilitat. En aquest sentit, s'ha observat que l'existència de deficiències i/o alteracions en diverses proteïnes, entre les quals destaquen la PLCζ, la IZUMO1 i la JUNO, és una causa d'infertilitat. Així doncs, hom podria utilitzar aquestes proteïnes crucials com a eines de prevenció i diagnòstic de la infertilitat associada als diferents processos moleculars descrits, alhora que aquestes podrien servir com a punt de partida per a l'estudi de la infertilitat idiopàtica. Tanmateix, i atès que en la majoria de casos la recerca encara es troba a les basseroles, calen més estudis tant en animals models com en l'espècie humana. En aquest darrer cas, hi ha una dificultat afegida com a conseqüència de la poca disponibilitat d'oòcits per a la recerca.

Resumen

La infertilidad afecta entre un 10 y un 15% de la población mundial actual, y se asocia tanto con desórdenes y alteraciones del sistema reproductor femenino como del masculino. Por otro lado, la infertilidad idiopática, esto es, aquella que no se puede atribuir ni al factor femenino ni al masculino, afecta aproximadamente a un 25% de las parejas infértiles en edad de reproducirse. Es en este contexto donde se centra el objetivo principal del trabajo, que es conocer los mecanismos moleculares involucrados en la interacción de los gametos. Este conocimiento podría servir como punto de partida para relacionar la deficiencia de estos mecanismos con la infertilidad.

Con este propósito, se ha efectuado una revisión sistemática de la literatura actual extraída de la plataforma PubMed. Esta revisión sistemática ha sido sometida a unos criterios de selección y a un análisis posterior de la calidad, con la finalidad de establecer una sistemática de inclusión/exclusión de los diferentes artículos. A partir de los artículos seleccionados, se ha estudiado el proceso de fecundación en los mamíferos. Este proceso consiste en un conjunto de etapas complejas donde distintas proteínas, tanto espermáticas como oocitarias, desarrollan roles esenciales que garantizan esta interacción gamética. Así pues, el estudio de la función y participación de dichas proteínas es fundamental para comprender y tratar la infertilidad idiopática.

Durante la reacción acrosómica que sufre el espermatozoide, que es un proceso indispensable para que éste adquiera la capacidad de fecundar el oocito, hay varias proteínas espermáticas, sobre todo la PKA y otras de la familia de las SNAREs que juegan un papel clave. Además, las enzimas hidrolíticas liberadas después de esta reacción acrosómica, como la acrosina, y los receptores de progesterona y de proteínas de la ZP son imprescindibles para que el espermatozoide pueda penetrar la ZP. Posteriormente, se produce la interacción y fusión de las membranas de los gametos, evento en el cual la proteína espermática IZUMO1 y la proteína oocitaria JUNO tienen una función preeminente. En este sentido, se ha observado que deficiencias en ambas proteínas imposibilita que la fusión de los gametos se produzca, lo que se asocia con problemas severos de infertilidad. Finalmente, tras la fusión de las membranas, se desencadenan una serie de oscilaciones de calcio citosólico que permiten la activación del ovocito. En este proceso, que es necesario para que el oocito concluya la segunda división meiótica, la proteína específica espermática PLCζ. tiene un rol fundamental. Sin embargo, parece que esta proteína no llevaría a cabo esta función en solitario, sino que hay otras como la PAWP que también podrían estar involucradas.

En definitiva, ampliar el conocimiento sobre las proteínas espermáticas y oocitarias que participan en los acontecimientos que se producen antes, durante y después de la

fecundación es necesario para comprender de qué manera la disfunción de los diferentes mecanismos moleculares involucrados en estos procesos están relacionados con la infertilidad. En este sentido, se ha observado que la existencia de deficiencias y/o alteraciones en varias proteínas, entre las cuales destacan la PLCζ, la IZUMO1 y la JUNO, es una causa de infertilidad. Así pues, se podrían utilizar estas proteínas cruciales como herramientas de prevención y diagnóstico de la infertilidad asociada a los diferentes procesos moleculares descritos, al mismo tiempo que éstas podrían servir como punto de partida para el estudio de la infertilidad idiopática. Sin embargo, y dado que en la mayoría de casos la investigación aún se encuentra en sus inicios, se necesitan más estudios tanto en animales modelo como en la especie humana. En este último caso, hay una dificultad añadida como consecuencia de la poca disponibilidad de oocitos para la investigación científica.

Abstract

Infertility currently affects between 10% and 15% of the population worldwide, and it is associated with both male and female reproductive system disorders and alterations. On the other hand, idiopathic infertility, which cannot be attributed to either male or female factors, affects approximately 25% of infertile couples during their reproductive age. In this context, this Dissertation is focused on understanding the molecular mechanisms involved in the interaction of the gametes. This knowledge could serve as a starting point to relate the deficiency of these mechanisms to infertility.

Following this objective, a systematic review of the current literature was carried out extracted using PubMed. This systematic review was subjected to specific selection criteria and subsequent quality analysis in order to establish the inclusion/exclusion of articles. From the selected articles, the process of fertilization in mammals was studied. This process consists of a set of complex stages in which different proteins, borne by both the sperm and the oocyte, play essential roles that warrant gamete interaction. Thus, studying the function and participation of these proteins is essential to understand and treat idiopathic infertility.

During sperm capacitation, which is an indispensable process to acquire the ability to fertilize the oocyte, several sperm proteins, especially PKA and others of the SNAREs family, play a key role. In addition, hydrolytic enzymes released upon the acrosomal reaction, such as acrosin, and sperm receptors for both progesterone and ZP proteins are essential to achieve the ZP penetration capability. Subsequently, the interaction and fusion of the gamete membranes takes place, an event in which the sperm-specific protein IZUMO1 and the oocyte-specific one JUNO are primarily involved. In this regard, it has been observed that deficiencies in both proteins make gamete fusion impossible, which is associated with severe infertility problems. Finally, after membrane fusion, a series of calcium oscillations in the cytosol are triggered allowing oocyte activation. In this process, which is necessary for the oocyte to complete the second meiotic division, the sperm-specific protein PLCζ plays a key role. However, it seems that this protein would not carry out this function alone, since other proteins such as PAWP could also be involved.

In conclusion, it is necessary to increase the knowledge of sperm and oocyte proteins involved in the events that occur before, during and after fertilization in order to understand how dysfunctions in the different molecular mechanisms involved in these processes are related to infertility. In this regard, deficiencies and/or alterations in several proteins, especially PLCζ, IZUMO1 and JUNO, have been found to be a cause of infertility. Thus, these crucial proteins could be used as tools for the prevention and

diagnosis of infertility associated with the different molecular processes described, as they could also serve as a starting point for the study of idiopathic infertility. Nevertheless, as research is still in its infancy, further studies involving animal models and human are much warranted. Related with this, it is worth mentioning that additional difficulties on human research exist due to the limited availability of oocyte for scientific investigation.

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1. Introduction

Infertility is considered as a disease by the World Health Organization (WHO) (Committee & Society, 2020; Poongothai & Gopenath, 2009) and is defined as a failure to conceive a successful pregnancy after one year of frequent and unprotected intercourse (Poongothai & Gopenath, 2009). Nowadays, infertility affects approximately between 10% and 15% of couples in the reproductive-age worldwide which represents more than 70 million couples globally (Jose-miller et al., 2007). For this reason, some regular studies suggest that infertility increases year by year becoming a common health problem (Ribas-Maynou & Yeste, 2020).

Many studies support that causes of infertility differ between developed and underdeveloped countries, the developed ones being where a higher number of infertile couples exists (Van Zandvoort et al., 2001). It has been argued that research on infertility is not a priority in underdeveloped countries where contagious infections and chronic diseases remain uncontrolled (Vayena et al., 2002). Moreover, infertility is also differentiated between primary and secondary causes (Yeste et al., 2015); the primary cause is related to the inability to ever become pregnant and the second cause refers to those who have delivered a child in the past but they have lost the ability to carry a successful pregnancy (Word Health Organization, 2021). The estimated percentages of primary causes range between 1% and 8% of the total population; approximately 35% of patients respond to secondary causes (Bechoua et al., 2016; Poongothai & Gopenath, 2009).

While the absence of pregnancy can be attributed to disorders in the female or male reproductive system, there is an unexplained infertility that affects around 25% of the unfertile couples (Allahbadia, 2016). When considering the developed countries population, male factors represent about 20-30% of infertility cases, and 20-35% is related to a female factor. Moreover, 25-40% cases of infertility have been associated with both components of the couple (Jose-miller et al., 2007). On the other hand, excluding physical causes, there is a significant condition of infertility associated to psychological distress and lifestyle factors, which are usually preventable in both sexes (Fidler & Bernstein, 1999). Current data show that for many couples, the inability to carry a pregnancy has been related to emotional stress (Gameiro et al., 2015), unhealthy habits such as an unbalanced diet, eating disorders, obesity, excessive exercise, nicotine and alcohol consumption, high caffeine levels, etc. (Palomba et al., 2018; Silvestris et al., 2019). Furthermore, some recent studies have associated environmental exposure to fertility reduction, due to spontaneous abortions and inferior semen quality (Gatimel et al., 2017).

In the case of women, infertility may involve a wide variety of disorders including ovary dysfunction (40%) such as polycystic ovarian syndrome or diminished ovarian reserve (Jose-miller et al., 2007); tubal factors (30% approximately); and uterine disorders, such as endometriosis (15%) (Vannuccini et al., 2016). Other potential causes of female infertility have been related to an advanced maternal age (Silvestris et al., 2019) and a previous bacterial infection caused by sexually transmitted diseases (STDs) where *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are the main pathogenic microorganisms (Parks & Peipert, 2018). Although female infertility may be attributed to a high number of factors, the causes still remain unknown in more than 15-30% of cases (Parks & Peipert, 2018).

Male infertility is a multifactorial disorder that has been related to a wide combination of causes, including abnormal sperm condition (30% of male infertility cases, approximately) (Jensen et al., 2009) and altered sperm transport (between 10-20%), such as epididymis diseases, erectile dysfunction or retrograde ejaculation (Jose-miller et al., 2007). Despite the fundamental role of sperm abnormalities, testicular cancer or any other testicular disorder have also been associated to a decrease in semen quality and consequently with the probability of conception (Jensen et al., 2009). Likewise, medication can also lead to alterations of semen quality (Fainberg & Kashanian, 2019). Moreover, some studies account for the importance of sperm DNA integrity, which is related to fertilization success, embryo development and even implantation (Ribas-maynou & Yeste, 2020; Robinson et al., 2012). For this reason, sperm DNA fragmentation has been purported to be a powerful tool for the diagnosis of male infertility (Fainberg & Kashanian, 2019). Despite the known factors underlying male infertility, about 50% of cases still remain unexplained (Poongothai & Gopenath, 2009).

Infertility calls for an urgent action because it is a disease that affects many couples and may be rescued in some cases (Poongothai & Gopenath, 2009). Treatment of infertility includes different medical strategies depending on the cause: drugs to stimulate ovulation, surgery and assisted reproductive technology (ART) (Copen & Stephen, 2014; Szamatowicz, 2016). Moreover, ART englobes all *in vitro* treatments that try to address the absence of pregnancy; the main three treatments are: intrauterine insemination (IUI), in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) (Ribas-Maynou & Yeste, 2020), the two latter being combined with embryo transfer (Adamson et al., 2009). The most updated figures indicate that IVF and ICSI are the most ART used. IVF is an extracorporeal fertilization, as sperm are placed in the vicinity of the oocyte, bind its vestments and fuse with the oolemma. In contrast, ICSI does not need a direct interaction between gametes, but rather a single spermatozoon is injected into the oocyte (Adamson et al., 2009). Furthermore, in the last decade, ART has increased significantly, and not only have these techniques been intended to infertile patients but also to those who choose

to freeze their gametes for a family planning option in the future (Rinaudo & Adeleye, 2020). As a result, current figures indicate that around 33% and 32% of pregnancies are achieved following ICSI and IVF, respectively (Ramadan et al., 2012).

Consequently, it is the intention of this Dissertation to describe, through a thorough, systematic review of the literature, the crucial role of molecular interactions between gametes. This knowledge will shed some light into how fertilization events take place and will allow opening up new research ventures addressing what may be beyond the infertility cases (about 30%) for which a clear etiology still remains unknown.

2. Objectives

The main aim of this Dissertation is to review the different molecular mechanisms involved in gamete interaction and oocyte activation, which are described herein as a support to understand and rescue idiopathic infertility associated with the dysfunction of these mechanisms. With this purpose, a systematic review of the scientific literature available from the PubMed database was conducted.

Following this main objective, the following specific objectives were set:

To describe the composition of ZP and the events that sperm undergo within the oviductal environment (i.e. sperm capacitation and acrosomal reaction), as this is needed, to understand the subsequent reproductive processes in which both gametes are involved.

To know the set of essential proteins involved in the interaction and fusion of gamete membranes as well as their contribution to fertilization.

To comprehend the main features of oocyte activation, including the involvement of sperm proteins and the oocyte machinery, and to set the link between oocyte activation deficiency and total fertilization failure, which underlies some cases of infertility.

3. Methodology

The work is based on a systematic review of the current literature about the molecular mechanisms involved in gamete interaction. This is crucial in order for us to understand, as much as possible, the whole process as well as to solve an important percentage of unexplained infertility cases.

All articles used in this work have been extracted from the PubMed database. This is a bioinformatics portal of the National Center for Biotechnology Information (NCBI) that amalgamates the abstracts of articles published in Journals indexed in SCI/JCR.

Advanced configuration of PubMed has been used in order to achieve the maximum accuracy for article selection. This advanced searching configuration allows for the selection of articles with pre-established keywords in the title or/and the abstract. Thus, combinations of the following selected keywords were searched: *infertility*, *oocyte*, *sperm*, *oocyte activation deficiency (OAD)*, *gamete fusion*, *fertilization*, *acrosome reaction*, *unexplained infertility*, *total fertilization failure*, and *rescue of failed oocyte activation*.

In order to carry out the search of articles, strict selection criteria were established (Fig. 1). Only articles published in English were chosen, whereas reviews, systematic reviews and meta-analysis were excluded; in the latter case, however, some references of these excluded items were used to identify valid articles for the research. Second, only articles treating infertility in humans, non-marsupial mammals of laboratory and farm animals were selected. As the last step, articles that contained information that was not relevant or decisive for the current study, despite containing the keywords in the title or abstract, were discarded. Moreover, articles selected in the previous points were further graded following NCBI Study Quality Assessment Tools (*https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools*). In order for an article to be included, it had to reach a quality score equal to or higher than 5. The other articles were excluded.

Finally, for citations and referencing, Mendeley management software was used, as this tool has the possibility of generating correct structure, orders references and is compliant with authors' rights.

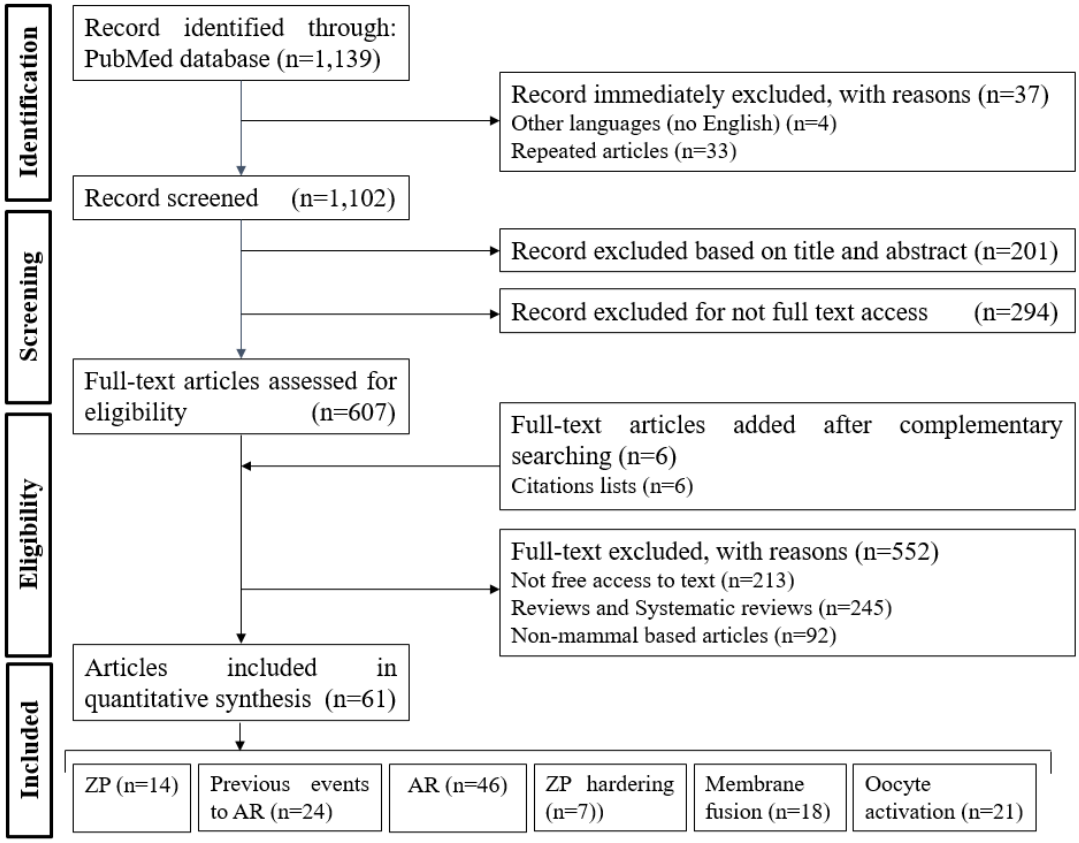

Figure 1 Flow chart of the literature search and selection criteria used in the present Dissertation.

3.1 Ethical and sustainability criteria

Standard ethical and environmental sustainability criteria were applied throughout this Dissertation. Briefly, only articles granted an approval from the corresponding Ethics Committee were selected and included. Furthermore, studies that were carried out with laboratory and production/domestic animals were only selected when they fulfilled with the 3R principle (*Replacement, Reduction and Refinement*). Furthermore, studies dealing with humans were only included when they explicitly stated that patient approval was obtained.

Moreover, copyright and authors' rights were always respected, and appropriate acknowledge to the authors of every article was given through citation and elaboration of a proper reference list.

Regarding the sustainability criteria, all selected articles were read electronically, avoiding paper waste. As laboratory entry was not required, since this is a systematic review, no biological or chemical reagents/materials were used.

4. Results

Mammalian fertilization consists of a serial of sequential events, initiated by sperm migration though the female reproductive tract. During their transit through that tract, sperm undergo capacitation, which is required for them to subsequently trigger acrosome reaction (AR) (Fara & Bar, 2016). Following this, sperm are able to interact with and penetrate the zona pellucida (ZP), then fuse with the oocyte membrane and finally activate the oocyte (Cheung et al., 2020; Hirose et al., 2020; Jean et al., 2018).

By going deeper into the different steps involved in the molecular mechanisms underlying the interaction between a spermatozoon and an oocyte, one may set a starting point for further research aimed at understanding and solving the unknown infertility associated to the dysfunction of these mechanisms.

4.1 The Zona Pellucida (ZP)

The following results of the ZP composition and its participation in mammalian fertilization are supported by 14 articles selected after undergoing the inclusion-exclusion processes listed in the methods section of this Dissertation. Moreover, and as aforementioned, articles were subjected to quality analysis after applying selection criteria. Only articles that exhibited a favorable score (Quality Score ≥ 5) were included.

Mammalian oocytes, including the human ones, are surrounded by a glycoprotein matrix called the Zona Pellucida (ZP), which protects the oocyte (Bielfeld et al., 1994; Paper et al., 2007; Tomes et al., 2002). This ZP matrix undergoes a series of changes during oocyte maturation, modifying a spongy appearance with numerous pores to a compact surface (Balbach et al., 2020; Watanabe & Kondoh, 2011). The composition and structure of this extracellular matrix varies across mammalian species (Hamze et al., 2019; Vandevoort et al., 2014). Thus, while some species, such as the pig and the mouse, present three types of ZP-glycoproteins, other, like humans, have four. Remarkably, the difference in the number of ZP-glycoproteins between species lies on the presence of ZP4 in humans, which is encoded by a pseudogene of *ZP3* in mice (Chiu et al., 2008). In spite of these differences, the functions of these proteins are similar in all mammals: protection of the oocyte and embryo, regulation of the sperm-oocyte interaction and prevention of polyspermy (Chiu et al., 2010).

While mounting evidence suggests that acrosome reaction is triggered in the oviduct before sperm interact with oocyte vestments (Buffone et al., 2016), ZP3-glycoprotein has

been consistently suggested to bind to its sperm receptor. In this sperm-ZP binding, which appears to be involved in the modulation of acrosome reaction, ZP4 also plays a role in humans, as it has been reported to aid ZP3 in sperm-oocyte interaction (Emiliozzi & Fenichel, 1997; Fukami et al., 2001; Tomes et al., 2002) . Subsequently, ZP2 interacts with the inner acrosome sperm membrane. Although ZP1 is not directly involved in sperm-ZP interaction, it participates in the maintenance of the structural integrity of the ZP matrix and, together with ZP2, hardens that extracellular matrix upon sperm-oocyte fusion, thus preventing polyspermy (Chiu et al., 2008; Ensslen et al., 2013; Hamze et al., 2019).

4.2 Previous events to the AR: Sperm capacitation and hyperactivation

The mammalian spermatozoon has to undergo capacitation and hyperactivation prior to being able to trigger the acrosome reaction; thus, both processes are crucial in order for a spermatozoon to successfully fertilize an oocyte (Breitbart, 2015). Thus, the following description of both processes is supported by 24 articles selected according to the methods section. Again, all articles were subjected to quality analysis, and only those showing favorable scores (Quality Score ≥ 5) were incorporated into the present study.

The principal previous event to achieve acrosome reaction (AR) is sperm capacitation which some authors have considered as the final maturation step that allows sperm to interact with the oocyte (Brucker et al., 1994). This process consists of a multiple structural changes to protein-lipid organization, fluidity and permeability of the plasma membrane, and occurs within the female reproductive tract (Battistone et al., 2013; Li et al., 2020; Tardif et al., 2001). Sperm capacitation also prepares the spermatozoon to undergo the AR (Bonaccorsi et al., 1995; Tomiyama et al., 1995).

The main changes in the sperm membrane consist of cholesterol depletion in the outer membrane. This efflux results in changes of membrane fluidity and induces the rearrangement of lipid rafts (Bronson et al., 1999; Nimlamool et al., 2013; Watanabe & Kondoh, 2011). While other molecular mechanisms involved in sperm capacitation are yet to be characterized (Ryu et al., 2014), cholesterol depletion from the sperm membrane is possible because the high concentration of albumin in the female reproductive tract acts as a cholesterol acceptor (Bonaccorsi et al., 1995; Emiliozzi & Fenichel, 1997; Visconti et al., 1995).

Moreover, to successfully transit through the female reproductive tract and fertilize an oocyte, sperm must first undergo a series of motility alterations. In this context, the transition from progressive motility (activated status) to a hyperactivated one is

indispensable for penetration of oocyte vestments and thus successful fertilization (Ogura et al., 2016; Visconti et al., 1995). Upon entering the female reproductive tract, sperm are exposed to high concentrations of bicarbonate which directly stimulates a soluble form of adenyl-cyclase (Breitbart, 2015; Megnagi et al., 2015; Xie et al., 2006). Thus, sperm motility is mostly regulated by both calcium influx and cAMP (Tardif et al., 2001; Wasco et al., 1989), the latter being the substrate of protein kinase A (PKA) which phosphorylates several sperm proteins (Xie et al., 2006). These changes in phosphorylation also regulate sperm motility, as well as intervene in the actin polymerization of the acrosomal region and the tail, helped by proteins such as gelosin or cofilin. Thus, not only is protein phosphorylation triggered by PKA crucial for regulating sperm motility, but it also modulates the sperm ability to trigger the acrosome reaction (Breitbart, 2015; Megnagi et al., 2015).

On the other hand, calcium is another secondary main messenger that is involved in many reproductive processes, such as sperm capacitation and the transition to a hyperactivated sperm motility (Chávez et al., 2018). In this regard, novel studies, such as that of Balbach et al. (2020), suggest that calcium selective channels in the flagellar membrane called CatSper might be the responsible for the massively entry of calcium during hyperactivation. In effect, CatSper channels detect pH variations and their opening allows high levels of calcium to enter the spermatozoon; this massive calcium influx is responsible for changes in motility patterns and capacitation-related events (Balbach et al., 2020; Chávez et al., 2018).

4.3 Acrosome reaction

Acrosome reaction is a critical step for a spermatozoon to fertilize an oocyte and is crucial for IVF protocols, that is why it has been extensively studied in the last five decades. Thus, this section is supported by 46 articles in total. After applying the different selection criteria and quality analysis, two articles were discarded due to a low-quality score (<5).

During late capacitation events, the outer acrosomal membrane is fused with the plasma membrane of the oocyte (Sosnik et al., 2009). When capacitated sperm are exposed to progesterone, acrosome exocytosis is triggered and enzymes, such as acrosin, are released; this allows for sperm penetration through ZP (Paper et al., 2007; Srivastava & Yanagimachi, 1986). Thus, only capacitated sperm that have completed the acrosome reaction are able to penetrate the ZP and subsequently fuse with the oocyte plasma membrane (Tomiyama et al., 1995).

The acrosome is a unique structure in the sperm head that is derived from the Golgi apparatus. Moreover, the acrosomal vesicle is known to confer an acidic pH environment (approximately 5.3) containing several hydrolytic enzymes, such as proteases and acrosin (Chávez et al., 2018). The function of these enzymes is ZP degradation, which then allows sperm to pass through that matrix (Ensslen et al., 2013; Komorowski et al., 2003). Furthermore, several proteins are involved in acrosome exocytosis; particularly, SNAREs, which are calcium-dependent, are vital for the fusion of plasma and outer acrosome membranes prior to exocytosis (Hao et al., 2014; Tomes et al., 2002).

Although the acrosome reaction allows for the penetration of ZP, some studies such as that of Bielfeld et al. (1994), suggest that it is initiated before sperm interact with the ZP. Concretely, mounting evidence suggests that the onset of acrosome reaction takes place in the middle of the isthmus of the oviduct rather than upon sperm binding to ZP surface. Around ovulation, the pH increases in the isthmus; this seems to trigger a signaling cascade that, together with a calcium peak, triggers the acrosome reaction (Hirose et al., 2020). Since data regarding these events are still inconsistent, further research on this realm is much warranted.

4.3.1 ZP-spermatozoon binding

Interaction of sperm with ZP has been under scrutiny and, thus far, different models have been proposed. The most accepted one, despite being the source of much debate, contemplates two sequential bindings. Primary binding involves the conversion of proacrosin into its active form, named acrosin (Chávez et al., 2018; Hirose et al., 2020). Proacrosin is the inactive form which is stored inside the acrosomal vesicle until the AR occurs; the active form called acrosin is a serine protease and plays an essential role in sperm penetration and ZP-sperm interaction (Chávez et al., 2018; Hirose et al., 2020).

Thus, the interaction between sperm and the ZP of the oocyte takes place in two steps. In the primary binding, multiple sperm receptors of ZP such as kinases, SP56, trypsin protein, ADAM3, etc. intervene; however, the exact role of these receptors is still uncertain (Alfieri et al., 2003; Kashir et al., 2011; Sachdeva et al., 2013). Subsequently, the secondary binding starts when the acrosome interacts with ZP glycoproteins, mainly ZP3, and this sperm-ZP interaction triggers a signaling cascade pathway that leads to the exocytosis of the acrosome (Emiliozzi & Fenichel, 1997; Fukami et al., 2001). This exocytosis involves the release of a large amount of hydrolytic enzymes, where acrosin has been thought to be a major player because of its strong hydrolyzing activity of ZP (Chávez et al., 2018; Chiu et al., 2008). Although the action mechanism of the complex signal transduction during acrosome reaction is uncertain, it is known that multiple factors

are involved in the exocytosis of the acrosomal contents. In effect, as the interaction between sperm and ZP appears to be insufficient to trigger the AR (Chiu et al., 2008), other acrosomal proteins and enzymes are also involved in the degradation of ZP (Chiu et al., 2008, 2010; Sosnik et al., 2009).

The ZP-sperm binding following acrosome reaction initiates a series of transduction pathways that results in the opening sperm calcium channels (Fig. 2), allowing the penetration and fusion of both gametes membranes (Bielfeld et al., 1994; Tomes et al., 1996). The first and small cytosolic calcium increase occurs when the spermatozoon interacts with the ZP. The ZP3 is the main glycoprotein which acts as a ligand for sperm membrane receptors and triggers calcium influx in human sperm, activating phospholipase C (Fukami et al., 2001) and regulating adenyl cyclase leading to an increase in cAMP levels (Chiu et al., 2010). Likewise, cAMP performs as a second messenger, ultimately activating protein kinase A (PKA) which results in the small cytosolic calcium increase (Li et al., 2020; Ogura et al., 2016; Visconti et al., 1995).

Moreover, this initial calcium influx activates phospholipase C gamma (PLCγ) (Tomes et al., 1996) generating diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3) from the hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP2), both being two major second messengers in the phosphoinositide pathway (Emiliozzi & Fenichel, 1997; Tarchala et al., 1995). DAG mediates the activation of protein kinase C (PKC) which, together with IP3, opens calcium channels in different sperm membrane regions, including plasma membrane and the outer acrosomal membrane (Fukami et al., 2001). Inositol 1,4,5-triphosphate activates Store Operated Calcium channels (SOC), which are located in the outer sperm membrane as shown in Fig. 2, and also mobilizes the calcium stored in the Endoplasmic Reticulum (ER) through the interaction with its receptor, IP3R (Fukami et al., 2001; Li et al., 2020). This second influx results in a massive and fast increase of the cytosolic calcium, which is crucial for triggering the final step of AR (Bielfeld et al., 1994; Chiu et al., 2010).

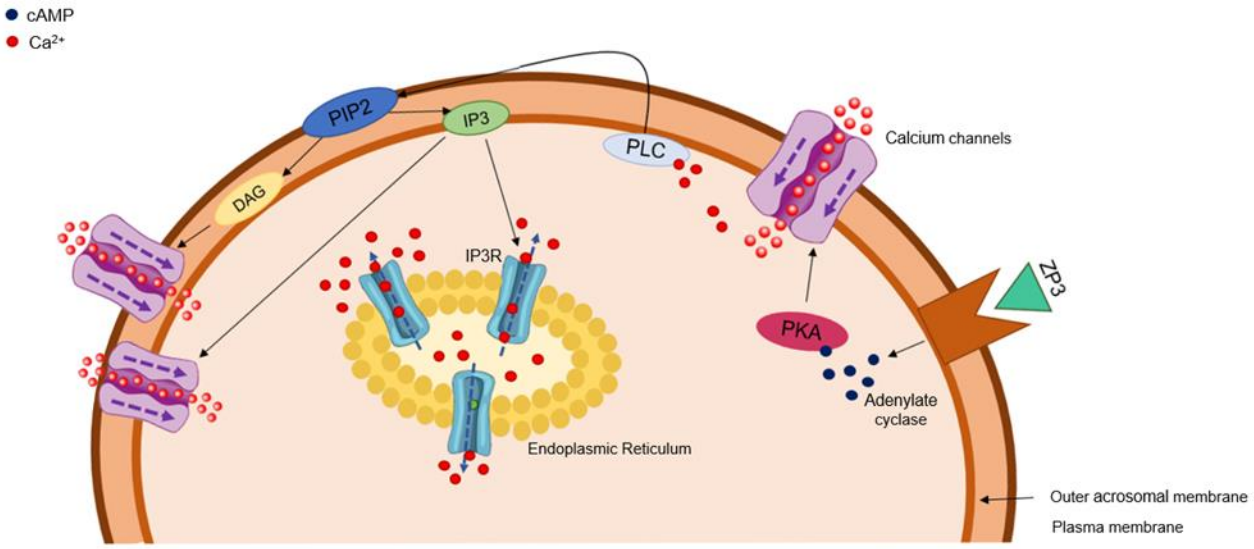

Figure 2 Classical Acrosome Reaction signaling pathway, led by cytosolic calcium influx. Upon sperm-ZP binding, through ZP3 glycoprotein, a small increase in cytosolic calcium occurs, leading adenylate cyclase to increase cAMP concentration, which activates PKA. This initial calcium influx activates a PLC, which hydrolyzes PIP2 into IP3 and DAG, opening different calcium channels. DAG regulates the opening of DAG in the plasma membrane and IP3 opens SOC, outer acrosomal membrane channels, and interacts with its receptor, IP3R, in the ER, triggering a massive calcium influx that leads to acrosome reaction. As stated in the text, this canonical mechanism is now under debate, since mounting evidence indicates that progesterone interacts with a receptor residing in sperm plasma membrane and this triggers AR. The interaction with sperm and progesterone would occur before the interaction of ZP3 with its sperm receptor.

ZP: Zona Pellucida; cAMP: cyclic adenosine monophosphate; PKA: protein kinase A; PLC: Phospholipase C; PIP2: phosphatidylinositol 4,5-biphosphate; IP3: inositol 1,4,5-triphosphate; DAG: diglycerol; SOC: Store Operated Calcium; ER: Endoplasmic Reticulum; IP3R: IP3 Receptor

It is important to note that all these possible interactions between sperm receptors and ZP-glycoproteins have been identified in mice, supported by the aforementioned studies, and have been extrapolated into humans. In this regard, all these data must be taken carefully, as one must assume that differences in the structure and function of ZP between mammalian species exist.

4.3.2 Inducers of the Acrosome Reaction

The oviductal environment is essential for AR induction in sperm, as the follicular fluid contains modulating substances that act as potent AR inducers in capacitated spermatozoa (Burrello et al., 2004; Paper et al., 2007). Previous studies showed that AR can be modulated and stimulated by multiple chemoattractive substances such as progesterone. This hormone, which is the main follicular fluid component, is released by cumulus cells

(Fukami et al., 2001; Schuffner et al., 2002). Moreover, it is known that progesterone does not induce the acrosome reaction in mammals unless the spermatozoon has undergone capacitation, acting as a filter to select only spermatozoa capable to penetrate the ZP (Bronson et al., 1999).

The sperm response to progesterone consists of an increase in intracellular calcium levels, which is thought to be an early step in the cascade of events leading to AR. Although the precise acting mechanism during the signal transduction pathways of progesterone remains unclear, the increase of free intracellular calcium has been recently related with GABA steroid receptors (Burrello et al., 2004; Fukami et al., 2001) and with the activation of a specific calcium sperm channel called CatSper (Chávez et al., 2018; Fara & Bar, 2016). While the exact activation mechanism of CatSper is still uncertain, there is evidence linking potential membrane depolarization and alkalization of intracellular pH to the activation and opening of these calcium channels (Balbach et al., 2020).

Other inducers present in the follicular fluid are estrogens (Ryu et al., 2014) and albumin, the latter being a key protein for stimulating capacitation in mammalian spermatozoa since it takes the cholesterol released from the sperm plasma membrane (Bonaccorsi et al., 1995; Visconti et al., 1995). Plasma membrane is composed of multiple lipids, and cholesterol is the major regulator of permeability and fluidity of plasma membrane (Khorasani et al., 2000), influencing the capacitation process and consequently the sperm ability to undergo to the AR (Bronson et al., 1999; Megnagi et al., 2015).

4.4 ZP hardening

The following brief approach to the process known as ZP-hardening and its corresponding contribution to polyspermy blockage is written under the analysis of seven selected articles with fair quality (Quality Score ≥ 7).

Sperm-oocyte binding induces rapid changes in the ZP matrix, which are crucial to prevent polyspermy (Kashir et al., 2012; Yeste, Jones, Amdani, Yelumalai, et al., 2016). This process, which is known as ZP hardening, transforms the matrix into a physical barrier with hard enzymatic digestion resilience (Balbach et al., 2020; Chalbi et al., 2014). Such a hardening occurs in the fertilized oocyte during the cortical reaction, and is triggered by the release of cortical granules, which contain proteases, such as metalloproteases (Ensslen et al., 2013; Komorowski et al., 2003). These proteases interact with ZP glycoproteins, mainly ZP2, and this triggers ZP hardening (Balbach et al., 2020).

Besides the major role played by ZP2 glycoprotein, ZP1 is also involved in the hardening process and consequently prevents polyspermy (Nomikos et al., 2013). Moreover, a protein named Fetuin-B (FETUB) is implied in the regulation of ZP hardening, as it inhibits premature ZP hardening and has been suggested to be useful for fertility preservation strategies (Ensslen et al., 2013).

4.5 Fusion between gamete membranes

In mammals, adhesion and fusion of sperm and oocyte plasma membranes are essential for fertilization which, as aforementioned, can only be possible after successful acrosome reaction (Nimlamool et al., 2013; Paper et al., 2007). During gamete fusion, the equatorial segment of the acrosome-reacted spermatozoon fuses with the oolemma, in a process that involves several protein, some of which have not yet been identified (Alfieri et al., 2003). Table 1 shows the principal proteins and the study of their molecular interactions will propel progress toward the understanding of sperm-oocyte fusion and the associated idiopathic infertility (Marcello et al., 2011; Noda et al., 2020).

The interaction between sperm and oocyte membranes during fertilization can be divided into two sequential steps; firstly, the spermatozoon binds the oocyte plasma membrane; secondly, membranes of the two gametes fuse (Chalbi et al., 2014). The results included in this section about the molecular mechanisms involved in the fusion of membranes between an oocyte and a spermatozoon are supported by 18 articles selected according to the inclusion or exclusion criteria exposed previously and obtaining, for all articles, a favorable score in relation to their quality (Quality Score ≥ 5).

4.5.1 Sperm proteins

From the sperm side, the principal proteins involved in gamete fusion are:

SPACA6 is localized in the equatorial segment (Ogura et al., 2016) and might be involved in the sperm fusion with the oolemma (Hagihara et al., 2015; Ogura et al., 2016). However, the exact molecular mechanism leading to gamete membrane fusion remains unknown, despite the fact that mice devoid of SPACA6 are sterile because of failure in sperm-oocyte fusion (Noda et al., 2020).

The SNARE complex plays an important role in membrane fusion during acrosomal exocytosis (Tomes et al., 2002), and it has shown that equatorin interacts helping one SNAREs family member (Alfieri et al., 2003; Hao et al., 2014).

CRISP (cysteine-rich secretory protein) is a component of acrosome and the outer dense fibers of the sperm tail (Nimlamool et al., 2013; Sachdeva et al., 2013). This protein has been described to modulate sperm flagellar motility and is also implied in membrane fusion, interacting with ZP glycoproteins, mainly ZP3 (Noda et al., 2020). Furthermore, reduced CRISP expression is observed in patients with asthenozoospermia, its levels being correlated with low sperm motility and abnormal morphology (Zhou et al., 2017).

ADAM family members interact with an oocyte integrin during membrane fusion (Alfieri et al., 2003; Sachdeva et al., 2013), but their action is also important in for ZP binding and oviduct migration (Komorowski et al., 2003; Marcello et al., 2011).

IZUMO1 is essential for sperm-oocyte fusion (Chalbi et al., 2014; Watanabe & Kondoh, 2011) and its oocyte receptor (JUNO) has also been identified (Balbach et al., 2020; Jean et al., 2018).

Despite all these sperm proteins for which a function has been purported, there is evidence that proteins, such as CD49 (Lee et al., 2015), SLLP1 (Sachdeva et al., 2013), SOF1 and TMEM95 (Noda et al., 2020), could also play some role and would also intervene in membrane fusion.

4.5.2 Oocyte proteins

There are multiple suggestions of oocyte proteins involved in adhesion and membrane fusion. The ones that have been thus far more consistently described to play a role are:

CD9, an integral membrane protein belonging to tetraspanin superfamily (TM4), which has been identified in mouse and human oocytes (Hamze et al., 2019; Jean et al., 2018). Although the molecular mechanism of sperm-oocyte interaction still remains unclear, the absence of CD9 from mouse oocytes leads to the loss of their ability to fuse with sperm membrane. This supports the function of this protein in oocyte fusion (Lee et al., 2015). Moreover, Alfieri et al. (2003) suggested that CD9 regulates the distribution of different oocyte membrane proteins, such as integrin, and creates solid focal adhesion contacts through the presence of a large extracellular loop that interacts with other proteins.

Another oocytes protein candidate is integrin, which is located in the oocyte membrane and interacts with sperm ADAM proteins (Sachdeva et al., 2013).

Another group of oocyte membrane proteins implicated in gamete fusion is GPI-Aps (Watanabe & Kondoh, 2011), which is a functionally diverse cluster of proteins that includes adhesion molecules, receptors, complement regulators, enzymes and signaling molecules (Alfieri et al., 2003).

Finally, JUNO is a membrane-tethered folate receptor also known as FOLR4 (Chalbi et al., 2014) that interacts with IZUMO1. Thus far, it is considered as one of the most important oocyte proteins involved in membrane fusion (Hagihara et al., 2015; Jean et al., 2018).

Table 1 Principal proteins involved in sperm-oocyte fusion and their implications for infertility

Sperm protein	*Oocyte protein*	*Interaction result*	*Effects of its absence on (in)fertility*	*References*
SPACA6	**Oolemma proteins**	Membrane fusion	SPACA6 absence leads to male infertility because of the sperm inability to fuse with the oolemma.	*Hagihara et al. (2015)* *Noda et al. (2020)* *Ogura et al. (2016)*
CRISP	**ZP3**	Sperm motility, membrane fusion and ZP interaction	No expression or reduced levels of CRISP are linked to a decrease in sperm motility and abnormal morphology. These alterations decrease sperm fertilizing ability.	*Burrello et al. (2004)* *Noda et al. (2020)* *Sachdeva et al. (2013)* *Zhou et al. (2017)*
ADAM	**Integrin**	Membrane fusion, ZP binding and oviduct migration	As an ADAM-null mouse model indicates, sperm devoid from ADAM are unable to bind the ZP. Furthermore, the absence of integrin in the oocyte membrane is also related with the inability of ZP to bind sperm, which leads to a reduction of female fertility	*Alfieri et al. (2003)* *Komorowski et al. (2003)* *Marcello et al. (2011)* *Sachdeva et al. (2013)*
IZUMO1	**JUNO**	Essential for membrane fusion and block of polyspermy	While sperm from an *Izumo1* KO mouse can penetrate the ZP, they accumulate in the perivitelline space and are not able to fuse with the oocyte, which makes them infertile. Moreover, the absence of JUNO affects female fertilization ability, as normal mature oocytes are formed but they cannot fuse with acrosome-reacted membrane sperm.	*Chalbi et al. (2014)* *Marcello et al. (2011)* *Hagihara et al. (2015)* *Hamze et al. (2019)* *Jean et al. (2018)* *Nimlamool et al. (2013)* *Noda et al. (2020)* *Ogura et al. (2016)* *Paper et al. (2007)* *Sosnik et al. (2009)* *Watanabe et al. (2011)*
CD49	**CD9**	Interaction between sperm and oocyte proteins which allows membranes fusion.	Although the molecular mechanisms of CD9-CD49 interaction remains unclear, female mice devoid of CD9 are sterile due to the loss of the membrane fusion ability. On the other hand, further studies to understand the exact role of CD49 in gamete fusion are required.	*Alfieri et al. (2003)* *Hagihara et al. (2015)* *Hamze et al. (2019)* *Jean et al. (2018)* *Lee et al. (2015)* *Noda et al. (2020)*

4.5.3 Interaction between IZUMO1 and JUNO

Sperm-oocyte fusion is indispensable for completing mammalian fertilization. Although the exact molecular mechanisms are poorly understood, the interaction between IZUMO1 and its oocyte receptor JUNO is one of the most studied and is known to be essential for gamete fusion (Hamze et al., 2019; Sachdeva et al., 2013), as well as in the blockade of polyspermy (Chalbi et al., 2014). Moreover, the crucial function of this binding has been reported to be conserved across mammalian species (Jean et al., 2018).

The main sperm protein conclusively demonstrated to be fundamental in sperm-oocyte fusion is IZUMO1, a member of the testis immunoglobulin superfamily type 1 (IgSF) protein (Sosnik et al., 2009) that is located in the acrosomal and equatorial regions of the head. Furthermore, it is highly conserved across mammalian species, and it is known that an IZUMO1 deficit in male mice causes infertility because these sperm are unable to fuse with the oocyte membrane (Jean et al., 2018; Noda et al., 2020; Yeste, Jones, Amdani, Yelumalai, et al., 2016).

During capacitation, there is a relocalization of IZUMO1 from the acrosome to the equatorial segment (Nimlamool et al., 2013; Ogura et al., 2016). This translocation is essential for gamete fusion and is regulated by a specific kinase named TSSK6 (testis-specific serine kinase 6) (Sosnik et al., 2009). TSSK6 is located in a posterior head region and has been suggested to be involved in the regulation of actin dynamics (Marcello et al., 2011). In fact, previous studies (Sosnik et al., 2009; Nimlamool et al., 2013) proved the important role of actin during relocalization of IZUMO1, due to the well-known involvement of the actin cytoskeleton in protein transport.

On the side of the female gamete, JUNO is the receptor of IZUMO1, which is a GPI-anchored protein located in the oocyte plasma membrane (Chalbi et al., 2014; Hagihara et al., 2015). As summarized in Table 1, the absence of JUNO leads to female infertility due to the formation of normal mature oocytes that cannot fuse with acrosome-reacted membrane sperm (Jean et al., 2018).

When IZUMO1-JUNO binding and interaction occurs, JUNO recognizes and associates selectively with monomeric IZUMO1 present in mature spermatozoa. Thereafter, the monomeric IZUMO1 form quickly dimerizes and allows establishing the tight adhesion of the two cell membranes (Hagihara et al., 2015; Sachdeva et al., 2013) (Fig. 3).

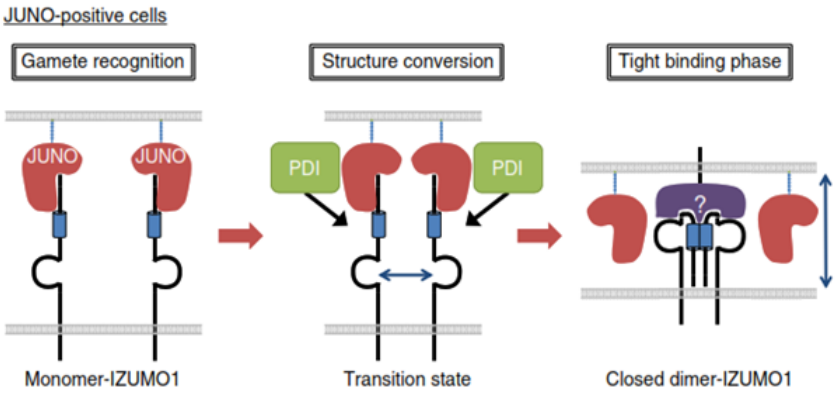

Figure 3 Modified from Hagihara et al. (2021)
IZUMO1-JUNO interaction. During gamete recognition, JUNO binds the monomeric IZUMO1 form; the accumulation of JUNO around the attached site of the spermatozoon increases the dimerization of IZUMO1, obtaining a closed-dimer IZUMO1 that confers the tight binding between both membranes.

Although CD9 is not essential for IZUMO1-JUNO binding, previous studies (Chalbi et al., 2014; Jean et al., 2018) observed that JUNO interacted with other proteins, such as CD9. While the exact reason of this extra-binding remains unknown, it might be another starting point for future studies of unexplained fertility.

4.6 Oocyte activation

Oocyte activation (OA) is the earliest step of embryo development after mammalian fertilization. Failure in this process leads to infertility that cannot be rescued with ICSI (Kashir et al., 2011, 2012; Nomikos et al., 2013). Thus, the following results explain the different molecular mechanisms involved in oocyte activation and are supported by 21 articles selected following the inclusion or exclusion criteria described in methods section and quality analysis (Quality Score obtained for 21 articles are ≥ 5).

Upon sperm-oocyte fusion, there is a series of calcium oscillations in the oocyte cytosol that alleviates this gamete from the Metaphase-II arrested. The oocyte then completes meiosis and initiates embryonic development (Ferrer-vaquer & Freour, 2016; Pan et al., 2013). Thus, calcium is a secondary messenger that plays a principal role in many reproductive processes such as sperm capacitation, AR and OA (Cheung et al., 2020).

The presence of sperm in the oocyte cytoplasm evokes a pattern of intracellular calcium oscillations that leads to a serial of crucial events classified as early and late events. Early events are membrane fusion; exocytosis of cortical granules, which is involved in ZP hardening and is crucial to block polyspermy; and decondensation of male chromatin (Nomikos et al., 2013; Yeste, Jones, Amdani, Yelumalai, et al., 2016). Late events are

initiated by the release of the oocyte from meiotic arrest and include decondensation of female chromatin, formation of the two pronuclei and recruitment of maternal mRNAs (Yeste, Jones, Amdani, Yelumalai, et al., 2016).

To delve into the possible causes of calcium oscillations, three principal theories were proposed (Kashir et al., 2011). The 'receptor theory' suggested an interaction between a sperm ligand and its corresponding oocyte receptor located in the oolemma; this binding would trigger the signal transduction pathway. Another hypothesis named 'calcium bomb' surmised that calcium enters directly into the oocyte via sperm plasma channels after gamete membrane fusion. In spite of this, these two theories were dismissed and the most currently accepted one is the 'sperm factor theory', which is supported by multiple studies and is based on a catalytic factor that is present in sperm head and initiates the aforementioned calcium oscillations.

4.6.1 Phospholipase C zeta, PLCζ

After membrane fusion, a series of calcium oscillations occurs in the oocyte cytosol (Kashir et al., 2012). The principal agent of triggering these calcium oscillations is a sperm-borne oocyte-activating factor (SOAF), which is in agreement with the 'sperm factor theory'. Mounting evidence identifies a sperm-specific protein phospholipase C zeta (PLCζ), which is localized along the inner acrosomal membrane and in the perinuclear theca (Nomikos et al., 2013; Yuan et al., 2020), as the SOAF. PLCζ is delivered by sperm head into the ooplasm, and catalyzes the hydrolysis of PIP2 (Chiu et al., 2010; Tomes et al., 1996). Hydrolyzation of PIP2 generates DAG and IP3 (Tarchala et al., 1995), which interacts with its receptor (IP3R) located in the endoplasmic reticulum (ER). The interaction of IP3 with its receptor triggers the characteristic cytoplasmic calcium release from internal ER calcium stores, which alleviates the oocyte from its MII-arrest (Fukami et al., 2001; Li et al., 2020). Moreover, not only does IP3 play a relevant role for mammalian OA, but it also participates in oocyte maturation and early embryo development (Emiliozzi & Fenichel, 1997).

Regarding the action of DAG, it works together with the calcium released from the ER activating a protein kinase C (PKC). This protein is translocated from the ooplasm to the oocyte cortex (Bielfeld et al., 1994), where participates in the exocytosis of cortical granules exocytosis and in the opening of calcium channels (Yeste, Jones, Amdani, Yelumalai, et al., 2016).

PLCζ is the smallest PLC isoform (Pan et al., 2013) and while it has been identified in different mammalian species, it has also been found in non-mammalian species

suggesting a role in the whole animal fertilization kingdom (Ferrer-vaquer & Freour, 2016; Kashir et al., 2011).

It is known that oocyte is arrested in Metaphase-II following ovulation, and the completion of the second meiotic division is mediated by PLCζ (Cheung et al., 2020; Nomikos et al., 2013). Thus, the anaphase-promoting complex (APC), which regulates reactivation of meiosis in the oocyte, is blocked by the early mitotic inhibitor 2 (EMI2) (Lee et al., 2015). Moreover, the characteristic calcium oscillations are sensed by a calcium-binding protein, calmodulin, which interacts with a kinase protein named Ca/calmodulin-dependent protein kinase II (CaMKII). CaMKII phosphorylates EMI2 allowing APC release, which degrades cyclin β, a subunit of the M-phase promoting factor (MPF). MPF is a heterodimer formed by two cyclines (cyclin β1 and CDK1) and is broken through the action of APC, thus allowing the reactivation of meiosis (Lee et al., 2015; Yeste, Jones, Amdani, Yelumalai, et al., 2016). (Fig. 4).

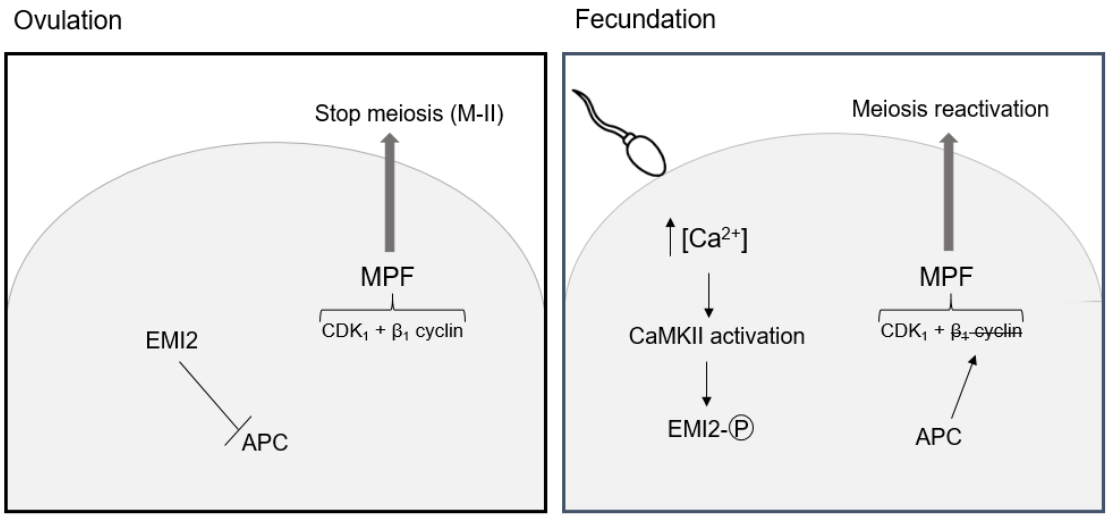

Figure 4 After ovulation, the oocyte is arrested at metaphase II because APC is blocked by EMI2, which cannot degrade MPC heterodimer, stunting the cell cycle. When a sperm cell fuses with the oocyte, a characteristic calcium oscillations pattern activates CaMKII, which phosphorylates EMI2, allowing the release of APC. APC degrades cyclin β_1 of MPC, thus alleviating the oocyte from Metaphase-II arrest.EMI2: early mitotic inhibitor 2; APC: anaphase-promoting complex; M-phase promoting factor; CDK1: Cyclin Dependent Kinase 1.

Despite most findings supporting that PLCζ is the major candidate to be the sperm-borne oocyte activation factor (SOAF) (Nomikos et al., 2013), some other proteins have also been suggested to be involved in the oocyte activation in mammals. One of these proteins is the post-acrosomal sheath WWP domain-binding protein (PAWP). This protein, also known as WP2, is exclusively located in the post-acrosomal region of the sperm perinuclear theca (Pan et al., 2013). While some evidence suggests its participation in

oocyte activation, the exact mechanism through which this protein plays such a role remains to be confirmed through further studies (Sanusi et al., 2015) (Table 2).

Table 2 PLCζ and PAWP and their mechanisms to activate the oocyte

Factor	*Localization*	*Contribution to the OA*	*References*
PLCζ	It is located along the inner acrosomal membrane as well as in the perinuclear theca region.	PLCζ hydrolyses PIP2 into IP3 and DAG, whose action triggers calcium oscillations in the oocyte.	*Cheung et al. (2020)* *Ferrer-vaquer & Freour (2016)* *Kashir et al. (2011)* *Nomikos te al. (2013)* *Pan et al. (2013)* *Sanusi et al. (2015)* *Yeste et al. (2016)* *Yuan et al. (2020)*
PAWP	PAWP has been proposed as a SOAF and it has been identified in the post-acrosomal sheath of sperm perinuclear theca.	The exact molecular mechanism through which PAWP would activate the oocyte remains unclear.	*Pan et al. (2013)* *Sanusi et al. (2015)*

PLCζ: phospholipase C zeta; PAWP: post-acrosomal WWP-domain binding protein; PIP2: phosphatidylinositol 4,5-biphosphate; IP3: inositol 1,4,5-triphosphate; DAG: diacylglycerol; SOAF: sperm-borne oocyte activation factor

5. Discussion

Firstly, it is worth noting that this Dissertation is supported by studies based on mammals, mainly mice (37% of the total articles) and humans (53% of the total articles), assuming the differences between both mammalian species, some of them have been previously explained. Therefore, the current information available for some of molecular processes involved in fertilization, which is the subject of the present systematic-review, is not entirely known and it should be confirmed in future studies.

Moreover, it is known, as explained above, that about 10-25% of infertility cases have no clear cause (Yeste, Jones, Amdani, Patel, et al., 2016). These cases have been suggested to be linked to deficiencies in the principal stages of fertilization, including sperm-oocyte fusion and activation of oocyte, where PLCζ, IZUMO1 and its receptor JUNO play a vital role (Chalbi et al., 2014; Kashir et al., 2011). Thus, IZUMO and JUNO are indispensable during human fertilization. As it has been mentioned previously and based on selected articles (Jean et al., 2018; Marcello et al., 2011), the absence of these two proteins clearly makes gamete membranes unable to fuse, stunting the fertilization process.

On the other side, infertility failure might be attributed to oocyte activation deficiency (OAD), which is the main factor explaining unsuccessful ICSI (1-5% of all treatment cycles; (Ferrer-vaquer & Freour, 2016; Sanusi et al., 2015). Although ART techniques are powerful tools to rescue infertility, oocyte quality and the sperm inability to activate the oocyte are considered the major reasons for total fertilization failure (TFF) (Kashir et al., 2012). PLCζ has been linked to oocyte activation deficiency (OAD) by the repeated failure of ICSI. In effect, the incapability of this sperm-specific protein to trigger calcium oscillations in the cytosol of the oocyte has been related to genetic disorders, e.g. globozoospermia, and abnormalities during spermatogenesis (Yuan et al., 2020). A study by Pan et al. (2013) identified the presence of two histidine mutations in *PLCZ1* gene in some infertile patients; histidine residues were substituted by a proline in the 398th position (H398P) and by a leucine in the position 233rd (H233L). These mutations underlid OAD and consequently led to TFF. In the light of all the aforementioned, PLCζ has been suggested as a valuable and novel clinical therapeutic agent for the diagnostic and treatment of OAD (Ferrer-vaquer & Freour, 2016). Thus, while the precise mechanism through which does PLCζ activate the oocyte is yet to be fully understood, evidence supports that it may be used as a therapeutic agent to overcome male infertility (Nomikos et al., 2013).

Moreover, despite the fact that the molecular processes leading to TFF are largely unknown, OAD is generally regarded as the main cause of fertilization failure (Yeste, Jones, Amdani, Yelumalai, et al., 2016). Thus, assisted oocyte activation (AOA) methods have been purported to trigger the calcium increase triggered by PLCζ, thus being able to rescue OAD in infertile couples. In addition, microinjection of cRNA encoding for *PLCZ1* or even that of the recombinant protein appear as more physiological alternatives to using calcium ionophores to treat OAD (Cheung et al., 2020; Kashir et al., 2011).

Therefore, gaining further knowledge on how crucial sperm and oocyte proteins, such as PLCζ, IZUMO1 and JUNO, participate in fertilization events may serve as a clinical tool for prognostic, diagnostic and therapy for infertility patients. Thus, this Dissertation provides updated data on those molecular mechanisms as well as discusses how do these proteins contribute to human fertilization. Given that research in this realm is still in its infancy and many data are needed to be elucidated, further investigations using animal models and, especially, human material - which particularly in the case of oocytes is much limited - are much warranted to understand the complex processes associated to idiopathic infertility.

6. Conclusions

Based on all the aforementioned, the following conclusions have been reached:

The current information available for the molecular mechanisms involved in gamete interaction and oocyte activation is still in the preliminary stages of research, which is why many of the data presented in this Dissertation must be corroborated with future studies in animal models and, above all, in humans. Moreover, the majority of proteins involved in gamete interaction have been identified in mice, and subsequently have been extrapolated to humans.

The information gathered from the different molecular processes involved in mammalian fertilization converges on calcium being an essential player in different signal transduction pathways (e.g. capacitation, acrosome reaction and oocyte activation).

Sperm and oocyte proteins involved in different essential stages of the gamete interaction process, such as ADAM, SP56, ZP3 glycoprotein, SNAREs, CD9, IZUMO1, PLCζ and JUNO, have been identified. Alterations, mutations and/or decreased levels of these proteins have been related to infertility. Therefore, the study of their status and function is herein proposed as a powerful key tool for the prognosis of infertile patients, and subsequently, for their therapy.

Continued research into the molecular mechanisms implied in gamete interaction and oocyte activation, including key proteins, might contribute to understand and solve idiopathic infertility.

7. El paper de la dona

En primer lloc, m'agradaria deixar per escrit que aquesta breu reflexió no deixa de ser un recull d'idees, pensaments i preguntes pròpies plantejades interiorment amb l'intent de plasmar-les en aquestes línies, i n'és aquest el motiu que per qualsevol possible controvèrsia o mala interpretació demano disculpes per endavant.

Així doncs, si be és cert que em considero una dona conscienciada sobretot en la lluita contra els estereotips, malauradament encara massa arrelats en la nostra societat, i en la igualtat d'oportunitats en l'àmbit laboral, submergint-me i ordenant pensaments, m'adono que durant els darrers anys universitaris no m'he aturat a reflexionar sobre el meu pas, com a dona, per ~~a~~ la universitat; i em sorprenc a mi mateixa. Per tant, aquest convit a la reflexió ofert pels meus mentors i mentores, estic segura que em podrà servir com a punt de partida per ajudar-me a conscienciar d'una manera més activa sobre una realitat que ens envolta i que sovint portem tant interioritzada que costa aturar-se a analitzar.

En el meu camí com a estudiant de la Facultat de Ciències, voldria dir que sempre m'he sentit molt acompanyada i recolzada, tant per part de les companyes i companys com també pel professorat, i m'agrada poder deixar constància que mai he presenciat cap acte de discriminació quant al gènere ni entre l'alumnat ni entre el professorat. Tanmateix, és cert que en la societat actual, desgraciadament, encara s'emmascaren molts estereotips sobre els rols i els comportaments estipulats segons el sexe, i extrapolant-ho a les converses diàries entre universitàries i universitaris, i jo, com una d'elles, segurament n'he estat responsable de l'ús d'alguns i víctima d'altres, pronunciats sense obscures intencions, sinó essent fruit d'una interiorització inconscient enmig d'una societat que tot i la seva lluita constant pel canvi i la igualtat, continua tenint arrelats aquests estereotips i conviccions de gènere sovint de manera subtil i difícil d'identificar.

D'altra banda, reflexionant sobre la participació de les dones en el món científic, i davant l'encara distribució desigual de l'alumnat en els diferents estudis, essent evident en graus com infermeria, enginyeries o magisteri, la curiositat m'ha fet indagar en els números pel que fa a la Facultat de Ciències i particularment al grau de Biologia. Així doncs, a través de les dades publicades al web de la Universitat de Girona, en l'apartat 'Igualtat de Gènere', he pogut confirmar que en ambdós casos, i des de que vaig trepitjar aquesta Universitat l'any 2016, les dones sempre han representat amb major nombre la facultat i el grau, i aquests valors s'han vist *in crescendo* en els últims anys (56% a la Facultat de Ciències i 60% en el grau de Biologia el curs 2019/20). Així doncs, podríem començar a intuir un major interès per una STEM (*Science, Technology, Engineering and Mathematics*) com la biologia per part de la comunitat femenina, que poc a poc va ocupant

i escalant posicions en el món científic-tecnològic, i, tot i que continua essent imprescindible la implementació, reivindicació i divulgació del paper de la dona en la ciència en les actuals i futures generacions, podríem celebrar, sense oblidar mai la nostra fita, una efímera victòria.

Analitzant el present treball, i començant per l'aspecte formal i de redacció del mateix, des d'un primer moment es va voler garantir un ús no sexista de la llengua, motiu pel qual, juntament amb el meu tutor, Marc Yeste, vàrem decidir redactar el treball amb anglès, ja que tot i la dificultat extra que aquest em podia suposar, al tractar-se d'una llengua que no fa diferència quant al gènere, vam evitar la sexualització d'aquesta, que en ocasions, encara que de manera inconscient, jo mateixa me n'atribueixo l'ús. D'altra banda, la determinació i posterior quantificació de dones en els diferents articles referenciats no ha estat possible, ja que en la majoria d'articles científics es signa a través del cognom i s'obvien els noms de pila dels investigadors i investigadores, fet que impossibilita determinar-ne el gènere.

TechnoSperm, el centre que m'ha acollit i assessorat per poder dur a terme el meu treball final de Grau és un Centre de recerca, innovació i transferència en biotecnologia de la reproducció animal i humana de la Universitat de Girona. El seu equip està format actualment per un total de 38 professionals i investigadors (s'inclouen els estudiants de grau i màster), on el gènere femení n'és el màxim representant, amb més del 75%.

En l'afany de satisfer una mica més la curiositat despertada, i després de comparar les dades anteriors amb altres centres i/o laboratoris de reproducció de Catalunya (*Dexeus, GiroFiv, IVF, CIRH, Teknon, FecunMed, FertiLab, Ueg, Girexx, Fiv Lleida* i *Embriogynes*) observo un domini femení del sector, en tots els centres anomenats l'equip de professionals està representat per més d'un 60% de dones.

I, seguidament em pregunto: per què aquest sector, relacionat amb la fertilitat i la reproducció, es troba tant feminitzat? Som les dones, les que sentim interès pels processos de gestació, reproducció i la infertilitat o són les profundes conviccions encara arrelades a la nostra societat en la que durant dècades s'ha atribuït una sèrie de característiques a les dones com el paper de cuidadora, sensibilitzada i conscienciada amb la gestació i la concepció dels fills?

Sigui quina sigui la resposta, el que en trec del cert, gràcies a aquesta reflexió, és que els estereotips i la implementació del rol de la dona en la societat durant dècades, continua encara present i determinant la distribució dels professionals en els diferents sectors laborals, i per tant, podria ser aquesta profunda i inconscient interiorització del paper femení, el que fa que el sector en el qual es basa el present treball, es trobi tant feminitzat. Tanmateix, sempre sota la meva humil opinió, creient en el poder de l'educació i de la

conscienciació com a eines per a poder-nos desmarcar d'aquesta imposició dels rols de gènere, el motiu per l'interès en una àrea de treball concreta no hauria de suposar, en cap cas, un mètode d'exclusió per a la seva dedicació, sobretot si aquesta està elegida sota una llibertat personal i lliurada de qualsevol temor inculcat pel terrorífic 'què es dirà', esdevenint, és per a mi, una tria totalment lícita.

D'altra banda, investigant un xic més els centres citats, i indagant en la direcció i fundació d'aquests, he pogut conèixer, a través de les dades publicades en les seves respectives plataformes digitals, que més del 80% dels laboratoris són dirigits per homes. Dades que em conviden de nou a preguntar-me'n el motiu, i retorno a aquesta llosa que tant ens pesa encara avui, el rol de la dona i aquestes atribucions passades, en què els homes simbolitzaven la fredor i la racionalitat, pròpia de grans empresaris i directius, i en canvi, les dones responien a una sensibilitat i un caràcter emocional, amb unes responsabilitats com a mare que les hi impossibilitava aconseguir alts càrrecs dins el món laboral. I tot i que, actualment, per la sort de tots i totes nosaltres, poderoses dones dirigeixen grans negocis, continua havent un forat negre en la repartició de les direccions i alts càrrecs segons el gènere. Tanmateix, sí que és cert, que els centres fundats més recentment, com són *Fertilab* o *Teknon – Reproducció assistida*, tenen la direcció sota mans femenines, fet que revela una petita llum esperançadora, llavor d'una lluita perseverant i continuada en l'abolició d'aquestes desigualtats i estereotips.

Així doncs, conscient de la importància d'aquesta lluita per quelcom tant simple com la igualtat d'oportunitats, sense tenir en compte quina és la parella de l'últim cromosoma i basant-nos exclusivament en la vàlua personal, acadèmica i laboral, com a únic criteri per a la posició de càrrecs laborals, m'agradaria acabar aquestes línies amb l'essència d'aquesta humil reflexió:

Primerament, agrair a tot l'equip de professionals del centre *TechnoSperm* la meva estada, no només en l'àmbit acadèmic, sinó també en el que a aquesta reflexió es refereix; durant la meva estada en el grup he percebut una ferma conscienciació respecte la perspectiva de gènere per part de tot l'equip, feta evident en les xerrades orientatives i de conscienciació organitzades per diferents investigadores i professores del grup, les quals m'han ajudat a sensibilitzar-me, i a qüestionar-me les meves pròpies idees.

D'altra banda, penso que en el nostre país, actualment ja no hi ha una prohibició directa que exclogui a les dones de certs mercats laborals i encaselli als homes en d'altres, però una interiorització durant dècades dels diferents rols segons el gènere, continuen sovint, de manera inconscient, determinant en molts sectors la presència i/o absència de les dones, que conjuntament amb subtils comentaris i estereotips extrets d'aquestes antigues conviccions, ens encarrilen a estudiar i posteriorment a laborar en àrees determinades. Però la lluita cap a la igualtat d'oportunitats està en marxa, i només a través d'unes eines

socials essencials i imprescindibles com l'educació, la reivindicació i sobretot la reflexió personal, obtindrem l'ajuda i la força per a guanyar la batalla. Convidar-nos sovint a la reflexió amb nosaltres mateixos, a qüestionar-nos el verdader motiu de perquè elegim el que elegim, en qualsevol aspecte, serà sempre la porta d'entrada al canvi per a la construcció d'una societat més justa i igualitària.

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