Review: The putative role of Progesterone Receptor membrane Component 1 in bovine oocyte development and competence

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ABSTRACT

Acquisition of developmental competence is a complex process in which many cell types cooperate to support oocyte maturation, fertilisation, and preimplantation embryonic development. In recent years, compelling evidence has shown that Progesterone Receptor Membra Component 1 (**PGRMC1**) is expressed in many cell types of the mammalian reproductive system where it exerts diverse functions. In the ovary, PGRMC1 affects follicular growth by controlling cell viability and proliferation of granulosa cells. PGRMC1 has also a direct role in promoting a proper completion of bovine oocyte maturation, as altering its function leads to defective chromosome segregation and polar body extrusion. Strikingly, the mechanism by which PGRMC1 controls mitotic and meiotic cell division seems to be conserved, involving an association with the spindle apparatus and the chromosomal passenger complex through Aurora kinase B. Conclusive data on a possible role of PGRMC1 in the preimplantation embryo are lacking and further research is needed to test whether the mechanisms that are set in place in mitotic cells also govern blastomere cleavage and subsequent differentiation. Finally, PGRMC1 is also expressed in oviductal cells and, as such, it might also impact fertilisation and early embryonic development, although this issue is completely unexplored. However, the study of PGRMC1 function in the mammalian reproductive system remains a complex matter, due to its pleiotropic function.

Implications

Progesterone Receptor membrane Component 1 is a multifunctional protein regulating multiple aspects of reproductive biology. Functional studies in which its expression has been experimentally reduced, support the hypothesis that it affects oocyte biology both extrinsically, through the regulation of follicular growth and intrinsically, during oocyte maturation. Evidence suggest that it also has a function in regulating early embryonic development. However, this hypothesis still needs to be confirmed. Here, we summarise current knowledge on putative mechanisms by which Progesterone Receptor membrane Component 1 controls oocyte development with the main aim of encouraging new research in this fascinating field.

Introduction

The development of a mature oocyte capable of being fertilised is a long journey that involves many diverse ovarian cells. Some of them are directly in contact with the oocyte, such as granulosa cells, while others, such as theca or luteal cells, regulate ovarian function and follicular growth, ultimately impacting on oocyte quality. Likewise, early embryo development involves various maternal tissues. These complex processes are the subjects of comprehensive textbooks (Hyttel et al., 2010; Hannon and Curry, 2018). However, before going into the topic of this review paper, a few basic concepts need to be recalled.

Oogenesis is deeply rooted into folliculogenesis. Oogenesis starts early during foetal development when the oogonia switch from mitotic to meiotic division. After the entry into meiosis, they become primary oocytes and arrest at the diplotene stage of prophase I, in the germ line cysts (Hyttel et al., 2010). Primordial follicle formation occurs when germ cell nests break apart, and individual oocytes become surrounded by a single layer of flat pregranulosa cells (O'Connell and Pepling, 2021). Folliculogenesis starts during foetal life and proceeds until the end of reproductive capacity. Specifically, follicle development begins with the initial recruitment of dormant primordial follicles and their activation

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when the oocyte initiates growth. Flat pregranulosa cells grow and differentiate becoming cuboidal granulosa cells, surrounding the oocyte in primary follicles (Ford et al., 2020). The growing primary follicle passes through the secondary stage, characterised by two or more layers of granulosa cells, the initial deposition of zona pellucida around the oocyte, and the beginning of theca cell layer formation. At this stage, the stromal cells surrounding the granulosa cells differentiate into an inner theca interna, a layer of steroid-producing cells cooperating with the granulosa cell, and the outer theca externa made of several layers of cells with supporting functions. When the secondary follicle transit to the tertiary stage, two types of granulosa cells differentiate, the mural granulosa cells, lining the follicle wall, and the cumulus granulosa cells, surrounding the oocyte and forming the cumulus cell-oocyte complex. As development continues, fluid-filled spaces emerge between the granulosa cells and coalesce into a single cavity, the antrum, characterising the tertiary follicle (Hyttel et al., 2010). The transition from secondary to tertiary follicles becomes progressively gonadotropin dependent, with cyclic recruitment of cohorts of follicles that brings to the development of the preovulatory follicle containing a mature oocyte able to be fertilised and become an embryo after ovulation and fertilisation (Hyttel et al., 1997; Hannon and Curry, 2018). After ovulation, the granulosa and the thecal cells differentiate into large and small luteal cells forming the Corpus Luteum, whose main function is secreting Progesterone (**P4**) (Hyttel et al., 2010). Fertilisation and first embryonic mitotic divisions (cleavage) occur in the oviduct, where epithelial oviductal cells exert a pivotal function. During cleavage, the overall volume of the embryo remains constant because the blastomere's cell division is not accompanied by an increase in the volume. Thereafter, as blastomeres continue to divide and differentiate, the embryo becomes a small cluster of cells, referred to as the morula and then a blastocyst, a fluid-filled structure in which the external cells constitute the trophectoderm and a small group of cells form the inner cell mass (Hyttel et al., 2010). The ability of an oocyte to become an embryo is generally referred to as "developmental competence".

Progesterone Receptor Membrane Component 1 (**PGRMC1**) is a multifunctional protein expressed in many of the above-described cells type of the reproductive tract, including granulosa cells and oocytes at different stages of folliculogenesis, as well as the preimplantation embryo (Peluso et al., 2006; Luciano et al., 2010; Aparicio et al., 2011; Luciano et al., 2011; Griffin et al., 2014; Ciesiolka et al., 2016; McCallum et al., 2016; Przygrodzka et al., 2016; Clark et al., 2017; Blaschka et al., 2019; Sang et al., 2021).

The multiplicity of biological systems and subcellular compartments in which PGRMC1 is expressed and the many proteins with which it forms complexes, likely account for its pleiotropic function. This complexity has been recently highlighted in major review papers (Cahill and Neubauer, 2021; Peluso and Pru, 2021; Cahill, 2022b; 2022a; Peluso, 2022; Pru, 2022) by scientists that have dedicated most of their scientific career to the understanding of this "enigmatic" protein, as it has been recently described (McGuire and Espenshade, 2022).

Among these functions, the ability to bind and mediate some of the P4 action has attracted the attention of most reproductive biologists. However, PGRMC1 also exerts P4's independent function that might be as well important in cellular biology. As such, PGRMC1 contributes to oocyte development and early embryogenesis in many ways.

In this review, we will summarise the knowledge of PGRMC1 function in all these cell types, specifically highlighting its putative role in oocyte and early embryos, where the experimental evidence available in the literature are still limited compared to the many studies carried out in follicular cells. When relevant, possible function in mediating progesterone activity will be presented and discussed. A schematic summary of the PGRMC1 function is shown in Fig. 1.
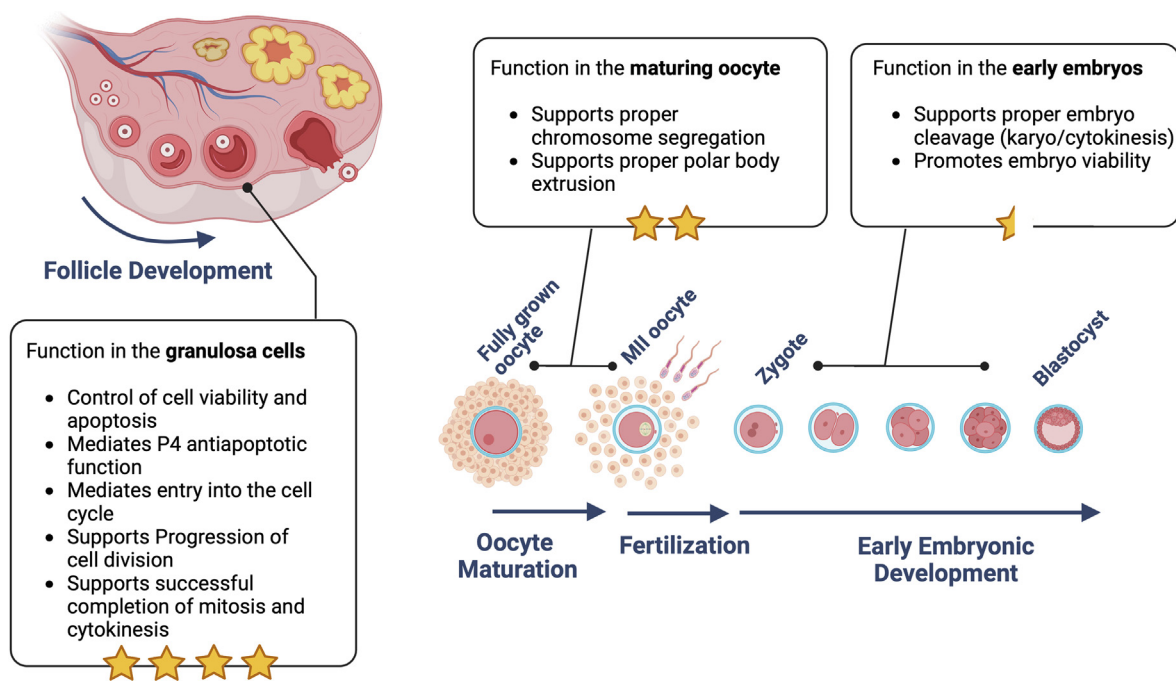

Fig. 1. Schematic representation of Progesterone Receptor Membrane Component 1 (PGRMC1) function in mammalian ovarian cells, oocytes and embryos. The number of stars in each box represents the robustness of the experimental data supporting the hypothetical function. Please refers to the text for specific bibliographic references. Figure created with BioRender.

The function of Progesterone Receptor Membra Component 1 in the follicular cells

Many studies have demonstrated that PGRMC family members (PGRMC1 and PGRMC2) participate in the control of cell viability, apoptosis, entry into the cell cycle and subsequent progression of cell division in ovarian cells, thus contributing to the regulation of mammalian ovarian follicle development. These studies have been accurately reviewed by Peluso and colleagues (Peluso, 2013; Peluso and Pru, 2014; Peluso, 2022), also in relation to the development of ovarian cancers (Peluso and Pru, 2021; Pru, 2022). Thus, in this paragraph, we will not go deeply into each of the studies, but rather summarise the most important concepts, as oocyte competence acquisition depends on follicle development. Firstly, though, two premises are necessary.

The first is that most of the above studies were designed to test whether PGRMC1 and two mediate some of the P4 non-canonical action in ovarian cells that could not be explained by the function of the classical nuclear P4 Receptor (**PGR**), either because they were too rapid, or because some cells do not express PGR (Peluso and Pru, 2014; Peluso, 2022). Therefore, the P4-independent function of PGRMC members might have been underestimated in follicular cells.

The second premise is that most conclusions are based on studies in rodents and human ovarian-derived cell lines. However, PGR expression in the follicle is species-specific. The intense research on 'alternative' P4's receptors was prompted by the evidence that, while P4 is known to slow follicular growth, PGR is not expressed in granulosa cells of growing follicles in mice, humans, rats and monkeys (reviewed in (Peluso and Pru, 2014)). On the contrary, PGR is only transiently expressed during the ovulatory luteinising hormone surge (Park and Mayo, 1991; Natraj and Richards, 1993; Park-Sarge and Mayo, 1994; Parksarge et al., 1995; Akison and Robker, 2012; Park et al., 2020; Peluso, 2022). Also, recent data have confirmed that, in mouse, PGR is only expressed in the granulosa cells of preovulatory follicles, but not in the cumulus cells nor in the oocyte (Smith et al., 2022). The same study has confirmed that PGR is required for follicle's wall rupture during ovulation, while follicular growth does not seem affected by the absence of PGR (Smith et al., 2022). These evidence led to the conclusion that P4's action on follicular growth and specifically on granulosa cells is mediated by other P4's binding proteins, including PGRMC1 and 2. However, this situation differs in ruminants. In sheep, PGR is expressed in granulosa cells of growing antral follicles (Ding et al., 2022), while in cattle, PGR is expressed in several ovarian cell types, including granulosa cells of primordial, primary, secondary and tertiary follicles (Van den Broeck et al., 2002; D'Haeseleer et al., 2007) as well as in both the cumulus cells and oocytes isolated from middle antral follicles (Aparicio et al., 2011; O'Shea et al., 2013). Accordingly, microarray analysis available in the opensource database EmbryoGene profiler revealed PGR mRNA expression in granulosa cells, including the cumulus cells, in various physiological contexts (https://emb-bioinfo.fsaa.ulaval.ca/IMAGE/index.html) (Sirard, 2014; Khan et al., 2016). Thus, considering PGRMC1 putative P4-dependent function in granulosa cells, there might be some differences between rodents and ruminants, because PGRMC1's function strictly depends on the expression of other P4 receptors, and binding partners. Interestingly, other progesterone receptors, such as members of the progestin AdipoQ receptors family, are expressed in granulosa cells and ovarian cell lines, which further complicate the dissection of the role of PGRMC1 in the ovarian follicle (Zhu et al., 2003; Peluso et al., 2009; Aparicio et al., 2011; Peluso and Pru, 2014; Kowalik et al., 2018; Medina-Laver et al., 2021; Thomas, 2022; Wendler and Wehling, 2022). In the following part of this paragraph, we will first summarise the findings in rodents and humans, and then highlight what it is known in cattle.

With the above consideration in mind, compelling data in various rodent and human ovarian cell lines support the hypothesis that PGRMC1 and PGRMC2 are key components of the machinery mediating P4's action (Peluso, 2022). Mechanistically, more data are available on PGRMC1 than on PGRMC2. A combination of experimental approaches has demonstrated that PGRMC1, but not PGRMC2, binds P4 (Kaluka et al. (2015) reviewed in Peluso (2022)). In addition, data have shown that (1) PGRMC1 has different sites of action within subcellular compartments including the plasma membrane and the intramembranous compartments, the cytoskeleton and the nucleus; (2) PGRMC1 is subjected to post-translational modifications, such as SUMOylation and phosphorylation, which likely influence its biology; (3) PGRMC1 directly or indirectly forms complexes with many essential proteins and factors, including various cytochrome P450 enzymes, EGF receptor PGRMC2, and, when expressed, PGR itself (reviewed in Peluso (2022)).

In addition, many studies in rat and human ovarian cell lines have shown that experimentally knocking down PGRMC1 reduced ovarian cell proliferation (Lodde and Peluso, 2011; Peluso et al., 2014; Peluso et al., 2019). However, cell growth occurs because of increased cell viability, which leads to reduced cell death in response to stressors or as the result of an increased frequency of mitotic cell divisions. PGRMC1, together with its partner PGRMC2, seems to participate in both processes.

Earlier studies focused on PGRMC1's ability to promote the viability of rat spontaneously immortalised granulosa cells (**SIGCS**) and human granulosa/luteal cell lines by mediating P4's antiapoptotic action induced by serum withdrawal, through the regulation, at least in part, of key genes of the apoptotic pathway (Peluso et al., 2008; Peluso et al., 2009; Peluso et al., 2010; Peluso, 2013; Peluso et al., 2013; Griffin et al., 2014). Lately, however, the focus has shifted to the control of cell division, specifically the entry into the cell cycle and the subsequent mitotic progression (reviewed in Peluso, 2013; Lodde et al., 2022; Peluso, 2022). In vitro, knocking down of PGRMC1 expression accelerates the rate of entry into the cell cycle SIGCs, through a complex mechanism ultimately involving NFKB/p65, and increases the percentage of mitotic figures (Peluso et al., 2019). However, this is not accompanied by an increase in cell proliferation, but rather block at the M-phase and cell death (Peluso et al., 2014; Peluso et al., 2019). The effect seen in mitotic cells is explained, at least in part, by PGRMC1's ability to localise to the mitotic spindle, where it interacts with ß-tubulin and controls microtubule stability, which in turn is mediated by P4, as shown in human cancer ovarian cell lines (Lodde and Peluso, 2011).

In mice, these in vitro studies were confirmed using a conditional KO model, where *Pgrmc1* was depleted in the follicular cells (Peluso, 2013; Peluso et al., 2019; Peluso, 2022). Strikingly, the number of antral follicles was significantly reduced in the homozygous *Pgrmc1* KO (Peluso, 2013). Furthermore, *Pgrmc1* KO was associated with an increased rate at which granulosa cells entered the cell cycle but, at the same time, undergo atresia, which was increased by $\geq$2-fold (Peluso et al., 2019; Peluso, 2022).

To the best of our knowledge, in the bovine species, the data supporting a role for PGRMC1 controlling follicular growth are restricted to the evidence that PGRMC1 is expressed in granulosa cells at all stages of folliculogenesis, and to functional in vitro studies in primary culture of bovine Granulosa cells (**bGCs**) (Luciano et al., 2011; Terzaghi et al., 2016).

These experiments tested the hypothesis that PGRMC1 is directly involved in the progression of mitotic cell division in bGC. Our data confirmed and expanded data in rat and human cell lines by showing that siRNA-mediated PGRMC1 knock-down reduced cell proliferation, while increasing the number of cells at

the G2/M−phase, which is in turn consistent with an arrested or prolonged M-phase (Terzaghi et al., 2016). Importantly, this observation was supported by time-lapse imaging revealing defects in mitotic progression when PGRMC1 is silenced and specifically during late karyokinesis (Terzaghi et al., 2016).

Furthermore, our studies revealed important insights into the mechanisms by which PGRMC1 mediates mitotic cell division through an interaction with Aurora Kinase B (**AURKB**). Precisely, immunofluorescence studies in bGC undergoing mitosis have shown that, as in other cell types, PGRMC1 associates with the spindle in metaphase, while it localises to the mid-zone and the mid-body in anaphase and telophase/cytokinesis (Luciano et al., 2010; Lodde and Peluso, 2011; Luciano et al., 2013; Juhlen et al., 2016; Luciano and Peluso, 2016; Terzaghi et al., 2016; Juhlen et al., 2018). At all these stages, in situ proximity ligation assay (**PLA**) revealed that PGRMC1 is in close proximity with AURKB. This finding suggests that PGRMC1 might mediate the action of the chromosomal passenger complex, particularly during cytokinesis because the interaction at the mid-body is most prominent when compared to other sites (Terzaghi et al., 2016). This observation is relevant since events occurring at the central spindle are crucial for proper cell division (Straight et al., 2003; Normand and King, 2010).

In addition, even though a direct function in the control of the mitotic spindle and other components of the cytoskeleton has not been yet functionally tested in cattle, we have recently hypothesised that interaction with the microtubules and other components of the actin cytoskeleton could be involved (Lodde et al., 2022). Likewise, interaction with clathrin and a putative involvement in the control of vesicle trafficking, which exerts pivotal function in cell division, might be also implicated (Cahill, 2020; Lodde et al., 2022).

Taken together, these observations support the hypothesis that in granulosa cells of different mammals, PGRMC1 supports successful completion of mitosis, avoiding the so-called "mitotic catastrophe" that results in cell death (Peluso and Pru, 2014; Terzaghi et al., 2016; Peluso, 2022). More precisely, the "mitotic catastrophe" is the '*atypical mechanism that senses mitotic failure and respond to it by driving the cell to an irreversible fate, be it apoptosis, necrosis or senescence*' (Kroemer et al., 2009; Vitale et al., 2011; Galluzzi et al., 2012).

To conclude this paragraph, is worth mentioning that an emerging aspect of PGRMC1 biology relates to a putative function in mediating ribogenesis and ribosome function (Terzaghi et al., 2018; Peluso and Pru, 2021; Pru, 2022), which might be a key aspect in ovarian cell proliferation and function. Clearly, more functional studies are needed to support this hypothesis.

The putative function of Progesterone Receptor Membra Component 1 in the maturing oocyte

As anticipated, the acquisition of developmental competence encompasses a plethora of yet-not-completely defined changes that affect the ability of the oocyte to be fertilised and support embryo and foetal development, eventually leading to a healthy pregnancy and delivery (Conti and Franciosi, 2018). However, the precise nature and timing of critical biological processes for developmental competence acquisition remain elusive in many aspects. For instance, it cannot be completely ruled out if events at the onset of primordial follicle activation, or at even earlier stages of oocyte specification and differentiation, may play a role. Nevertheless, during **oocyte growth** – corresponding to the stages from primary to Graafian follicles – and later, during **oocyte maturation** – in the preovulatory follicle - the oocyte undergoes a series of biochemical and structural changes in response to cell-autonomous signals and microenvironmental cues that collectively endow the

gamete with meiotic and developmental competence (Anderson and Albertini, 1976; Luciano et al., 2012; Conti and Franciosi, 2018). While the previous section considered the 'extrinsic' role of PGRMC1 in affecting the oocyte's ability to become an embryo, given its functions in the follicular niche that nurtures the oocyte, the current section aims at recapitulating the role of oocyte-intrinsic PGRMC1. To this extent, first, an overview of the putative roles of the protein during maturation will be given since they are better characterised compared to the functions that PGRMC1 might play during oocyte growth. The latter(s) will only be briefly presented since they are still largely speculative. Although this distinction may sound rather scholastic, it should be noted that oocyte maturation occurs in the virtual absence of transcription (Fair et al., 1995; De La Fuente and Eppig, 2001), therefore, the mechanisms in place act at non-genomic levels. Conversely, the extended growth phase of the oocyte is characterised by intense *de novo* RNA transcription (Moore and Lintern-Moore, 1978; Fair et al., 1995; Lodde et al., 2008), hence, genomic mechanisms are also expected at this stage.

Oocyte maturation is identified as the window of the meiotic division that goes from cell cycle re-entry, macroscopically identified with the dissolution of the nuclear envelop, to the metaphase of meiosis II (**MII**), marked by the extrusion of the first polar body. The faithful completion of this process leads to the ovulation of a fertilisable oocyte and, although in mammals, it occurs physiologically in response to the surge in luteinising hormone, it can also be recapitulated in vitro by removing from the inhibitory environment of the antral follicle the oocytes that have completed the growing phase (Moore and Lintern-Moore, 1978; Zhang et al., 2010). This so-called in vitro maturation (IVM) is the main experimental model used to study PGRMC1 function during oocyte maturation.

The current knowledge on PGRMC1's putative function(s) during mitotic and meiotic cell division has been traced in a recently published review, highlighting the multiple activities and mode of actions of the protein (Lodde et al., 2022). According to the conducted analyses and available data, PGRMC1 participates in key mechanisms that control meiosis progression, such as formation of the spindle, stabilisation of cytoskeletal elements, faithful separation of bivalent chromosomes, and cytokinesis, and, as such, are essential for proper oocyte maturation. As already mentioned, the pleiotropic nature of PGRMC1 is well established and likely regulated by the association to various molecules and complexes, such as, but not limited to, haem occupancy and interaction with cytochrome P450, formation of multiprotein complexes, subcellular compartmentalisation, and post-translational modifications (as recently reviewed by (Cahill, 2022b)). In this context, oocyte maturation is no exception.

One of the key events for the formation of a competent oocyte is the correct segregation of the genetic material, also identified as nuclear maturation. To this extent, the bivalent chromosomes must be separated between the secondary oocyte – the egg – and the first polar body (**PBI**) so that each contains one copy of the bivalent chromosomes, that are in turn made up of a pair of sister chromatids (Sanders and Jones, 2018). Euploidy is ensured by the separation of homologous chromosomes and retainment of cohesion between sister chromatids, while chromosomal missegregation generates aneuploidy, that is in most cases, incompatible with normal embryo development or cause of genetic syndromes (Charalambous et al., 2023). This process is the result of the formation of a bipolar spindle, the attachment of the chromosomes' kinetochores to the spindle microtubule, the separation of homologous chromosomes by the Anaphase-Promoting Complex (**APC**), and the final abscission (Ruchaud et al., 2007; Kitagawa and Lee, 2015), and it is overseen by the spindle assembly checkpoint (**SAC**), a multiprotein complex centred in the kinetochores (reviewed in (Mihajlovic and FitzHarris, 2018)).

PGRMC1 has been localised at the kinetochores of metaphasic chromosomes in bovine oocytes in association with one of the 'core' SAC proteins, AURKB (Luciano et al., 2010), leading to hypothesise a role for PGRMC1 in the mechanism of recognition and bi-orientation of the chromosome on the metaphasic plate. This hypothesis was confirmed using the loss of function experimental approaches whereby an anti-PGRMC1 blocking antibody or anti-PGRMC1 siRNA were injected into the ooplasm, with consequent observation of chromosomal scattering and formation of aberrant meiotic figures (Luciano et al., 2010; Terzaghi et al., 2016). Additional supporting evidence were provided by the observation that naturally occurring as well as pharmacologically induced displacement of AURKB were associated with mislocalisation of PGRMC1 (Luciano et al., 2013). Notably, the oocyte category carrying abnormal PGRMC1 and AURKB localisation also had a higher incidence of aneuploidy (Luciano et al., 2013), in line with the hypothesised role of PGRMC1 in chromosome segregation, and possibly providing a mechanistical explanation for their lowered developmental competence (Lodde et al., 2021).

Besides kinetochore occupancy in metaphasic chromosomes, in maturing bovine oocytes, PGRMC1 continued to co-localise with AURKB, and more specifically with its phosphorylated, active form (phospho-Thr232), during the karyo/cytokinesis steps. To this extent, dissociation from the chromosomes and concentration in the mid-zone and mid-body of the separating bivalent chromosome was shown by immunofluorescence and confocal microscopy (Luciano et al., 2010). This observation led to hypothesise that the PGRMC1 role extends to Anaphase I (AI) and Telophase I (TI), during which it associates with key components of the chromosome passenger complex in the mid-zone and of the furrow that divides the secondary oocyte from the PBI. The hypothesis of a participation of PGRMC1 in late events of karyo/cytokinesis was again experimentally addressed by siRNA injection in oocytes that were then in vitro matured. Indeed, besides the increased number of aberrant meiotic figures already reported above, the rate of PBI extrusion was decreased, somehow recapitulating the failed cytodieresis phenotype found in cultured bGC (Terzaghi et al., 2016). As a word of caution, the oocyte phenotype was less penetrant compared to the one observed in somatic cells, but there is reason to believe that the impossibility of fully depleting the oocyte of PGRMC1 protein by siRNA can itself account for the partial effect. It is likely that more incisive methodologies of protein depletion, such as morpholino oligonucleotides or TRIM away, might give more compelling outcomes. On this line of reasoning, when treating the oocytes with increasing doses of the PGRMC1 inhibitor AG-205, PBI extrusion was proportionally repressed, without interfering with the early stages of meiotic resumption (Terzaghi et al., 2016). Even though the specificity of AG-205 has been convincingly questioned (Cahill and Neubauer, 2021; Cahill, 2022b), the results obtained in the AG-205-treated oocytes seem to corroborate the role of PGRMC1 in the late stages of oocyte maturation, as they are consistent with PGRMC1 immunolocalisation and protein knockdown experiments. Clearly, more confirmatory experiments are needed to fully elucidate the putative off-target effects of AG-205 in mammalian oocytes.

From a mechanistic standpoint, how PGRMC1 participates in the maturation-related processes remains elusive and besides association with AURKB, other mechanisms have not been investigated in the oocyte. However, interactions with other cytoskeletal elements, as reported in somatic cells, are not apparent, at least in bovine oocytes, leaving the participation of PGRMC1 to the functions of the chromosome passenger complex, and meiotic furrow the best characterised thus far. Finally, whether PGRMC1 associates with these molecular complexes directly or indirectly via intracellular vesicles is currently not known.

After having described PGRMC1's roles in nuclear maturation of the oocytes, it should be remembered that cytoplasmic maturation is also necessary for producing a competent egg. Signs of cytoplasmic maturation are, for instance, the redistribution of organelles in specific ooplasmic compartments or changes in their shape and diffusion (Reader et al., 2017). Given the function of PGRMC1 in membrane vesicle trafficking, one would predict that this protein also participates in the regulation of such events. Nevertheless, this aspect of PGRMC1 biology has not been pursued thus far in relation to oocyte maturation.

In addition to organelles redistribution, cytoplasmic maturation consists of molecular and biochemical changes required for the formation of a good-quality oocyte. Given the virtual absence of ongoing transcription, regulation of the translation of previously stored mRNAs has been recently brought up as a key component of developmental competence (Franciosi et al., 2016; Conti and Franciosi, 2018). Notably, in PGRMC1 pull-down experiments, eukaryotic ribosomal proteins were isolated from the lysate of somatic cells (Peluso and Pru, 2021), leading us to hypothesise that PGRMC1 might be involved in the control of translation. Whether PGRMC1 has a similar function also in the oocyte has not been investigated. To this extent, it should be remembered that a great deal of the basic knowledge on the regulation of maternal mRNA translation derives from studies on Xenopus oocytes (Conti et al., 2016). In this animal model, meiotic re-entry is triggered by P4 exposure that, through a non-genomic mechanism, induces de novo protein translation necessary to induce the activation of the M-phase promoting factor (Meneau et al., 2020). Although extremely speculative, one might wonder if P4-related signalling cascades regulating mRNA translation have been evolutionarily conserved, to a certain extent, in mammals. However, the only piece of data available thus far that might link translation control in the oocyte and PGRMC1 is the localisation of the protein in the nucleolus (nucleolar-like body) of the female gamete (Terzaghi et al., 2018), where it might participate in ribogenesis, as recently suggested (Peluso and Pru, 2021; Cahill, 2022b). In such scenario, the PGRMC1 activity would fall into the uncharted area of the PGRMC1 functions during oocyte growth.

Even though PGRMC1 protein is strongly expressed in the nucleoplasm of the bovine oocytes at all stages of folliculogenesis (Luciano et al., 2011), its function during extended oogenesis has not been investigated yet. In somatic cells, genomic activities of the sumoylated PGRMC1 isoform have been described to act on promoters' regions of the transcription factor T-cell factor/lymphoid enhancer-binding factor (Peluso et al., 2012). Additionally, phosphorylated isoforms of PGRMC1 participate in the epigenetic regulation of cancer cells by acting on the DNA methylation levels at CpG islands (Thejer et al., 2020b). Immunolocalisation experiments conducted on ovarian sections did not show an apparent association of PGRMC1 with the oocyte chromatin, therefore, if genomic and epigenetic functions are retained during oocyte growth, an indirect interaction with the DNA might seem more plausible, similar to the mechanism suggested in Peluso and Pru (2021).

While the discussion on maturing oocytes was based on findings in in vitro matured bovine oocytes, and especially on studies that manipulated the PGRMC1 protein content using knock-down approaches, the role of PGRMC1 during oogenesis will be better addressed using germ line-specific genetic models, similar for instance to the approach used to deplete Pgrmc1 selectively from granulosa cells (Peluso et al., 2019). Given that the floxed Pgrmc1 allele is available, it should not be hard to obtain the Pgrmc1 deletion by crossing with mouse carrying the appropriate Cre-recombinase driver. Additionally, the use of Gdf9 or Zp3 drivers shall allow the understanding if oocyte-specific PGRMC1 plays a

role in primordial follicle activation and therefore addressing the involvement of PGRMC1 in primary ovarian failure, reported several times (Mansouri et al., 2008; van Dooren et al., 2009; Akison and Robker, 2012; Peluso, 2013; Peluso and Pru, 2014; Qin et al., 2015; Peluso et al., 2018; Venturella et al., 2019). However, given the functional overlap between PGRMC1 and PGRMC2, the other member of the PGRMC family, or other P4 effectors, the development of double or triple mutants might prove necessary. Hence, it cannot be excluded that studies on PGRMC1 conditional knockout models have not been reported thus far for this reason or that other issues may arise from the residual functional element obtained using the Cre/Lox system, as hypothesised in (Cahill, 2022b). However, it is interesting to note here that at the stage of primordial follicle formation, progesterone affects ovarian primordial follicle assembly (Nilsson and Skinner, 2009). In particular, the decrease of progesterone and oestrogen concentrations during mid-gestation was correlated with the primordial follicle assembly, while a high concentration in cultured foetal bovine ovaries significantly decreased follicle assembly (Nilsson and Skinner, 2009), similar to cultured mouse and rat ovaries (Kezele and Skinner, 2003; Chen et al., 2007). Surprisingly, in rat and bovine, the transcriptomic analysis indicated that PGR was not expressed at detectable levels, while progesterone receptors expressed at the time of follicle assembly comprised PGRMC1 (Nilsson et al., 2006; Nilsson and Skinner, 2009). It was suggested that low progesterone levels might support continued follicle development, while high levels inhibit follicle assembly. However, as already highlighted, the specific action of PGRMC1 remains to be explored at this stage.

A full *Pgrmc1* knockout mouse has been created by TALEN to study non-alcoholic fatty liver disease (Lee et al., 2018). However, fertility-related phenotypes are not reported, and they would eventually fail to address which biological compartment(s) in the hypothalamic-hypophysis-gonadal axis were mainly involved. Reduced or impaired fertility was instead described following the deletion of *pgrmc1* and/or *pgrmc2* in zebrafish (Aizen et al., 2018; Wu et al., 2018; Wu and Zhu, 2020). Although promising, these findings should be taken with caution, since also in this case, the deletion is not germ line-specific and, similarly to what was described in the frog, oocyte maturation is steroid-induced in zebrafish (Lessman, 2009). Conversely, P4 is not involved in the resumption of meiosis in mammalian oocytes.

Whether PGRMC1 effects during oocyte growth and maturation are, at least in part, triggered by P4 is not clear. Work conducted, once again, in bovine in vitro matured oocytes, suggests that P4 produced by the cumulus cells during oocyte maturation can positively affect the developmental competence (Aparicio et al., 2011). This effect however does not seem to be mediated by PGRMC1 or other non-genomic P4 receptors expressed in the cumulus-oocyte complex, but rather by nuclear PR, as treatment with its inhibitor RU 486 decreased the blastocyst yield. As a consequence, the putative P4 activity affecting the developmental competence is more likely exerted indirectly on the cumulus cells that are transcriptionally active and therefore capable of responding to nuclear PR activation. Further studies are still needed to address whether PGRMC1 acts as an effector of P4, keeping in mind that, in line with its pleiotropic nature, some functions might be controlled by P4 while others independent.

preimplantation embryo (Luciano et al., 2011; Sang et al., 2021). Interestingly, in the bovine morula, *PGRMC1* is one of the most abundant transcripts among those encoding for receptor genes using small molecules as ligands (Sang et al., 2021). Similar high transcript levels were reported for *PGRMC1* and two in porcine embryos (Cho et al., 2020). Moreover, in cattle, preliminary microarray data revealed that the abundance of PGRMC1 transcript varies significantly in in vivo versus in vitro-produced bovine embryos (https://emb-bioinfo.fsaa.ulaval.ca/IMAGE/cgi-bin/DoProfile.cgi?gene=PGRMC1&tissue=blastocysts). In the bovine zygote and blastocyst, PGRMC1 subcellular localisation mirrors the nuclear and cytoplasmic localisation seen in somatic cells (Luciano et al., 2011; Terzaghi et al., 2016; Terzaghi et al., 2018). However, these expression studies do not demonstrate that PGRMC1 regulates embryo function and more mechanistic studies are required.

In pigs, the function of PGRMC1 has been studied by treating in vitro-produced embryos with increasing concentration of the small molecule AG-205, which has been used as PGRMC1 antagonist, and assessing the effect on blastocyst formation and blastomere's apoptotic rate (Cho et al., 2020). These studies revealed that high concentrations of AG-205 (15 and 50 µM) were detrimental to embryo development, possibly due to toxic effects, while 5 µM concentration reduced the percentage of embryo reaching the blastocyst stage. On the contrary, lower doses (1 and 2 µM) did not reduce blastocyst formation but overall reduced the number of cells per embryo and increased the percentage of apoptotic cells (Cho et al., 2020). These effects were partially rescued by concomitant supplementation of 100 mM P4, confirming the protective effect of P4 treatment (Cho et al., 2020).

Given the emerging role of PGRMC1 in regulating cell division (Lodde et al., 2022), preliminary experiments were designed in our laboratory to assess PGRMC1 function during cleavage of the bovine embryo. Since modulation of gene expression in the bovine model is difficult to perform, we started to address the issue by treating in vitro-produced zygotes with increasing doses of AG-205. As shown in Fig. 2, high doses of AG-205 significantly increased the percentage of abnormal blastomeres showing multinucleated or anucleated blastomeres, which is consistent with the defective karyo/cytokinesis observed in granulosa cells and oocytes (Terzaghi et al., 2016). However, the effect of AG-205 was not reversed by treatment with 1 µM P4.

The main limitation of the above studies is that PGRMC1 function was solely perturbed by the small molecule AG-205. In fact, AG-205's specificity has been questioned lately. Increasing amount of data indicate that it has PGRMC-independent effect (Cahill, 2022b). Thus, the above data should be further validated to precisely dissect the role of PGRMC1 during early embryogenesis. Nevertheless, cytokinesis defects are consistent with pull-down experiments showing that AG-205 affects cytoskeletal components, including the ones that associate with PGRMC1 and with recent insights that PGRMC1 affects cell division through interactions/regulation of the cytoskeleton and possibly endovesicle trafficking machinery (Salsano et al., 2020; Teakel et al., 2020; Thejer et al., 2020a; Cahill and Neubauer, 2021; Lodde et al., 2022). Unfortunately, no specific PGRMC1 inhibitor is currently available to date. As such, CRISPR/cas9 technology (Lamas-Toranzo et al., 2018) and the newly developed trim-away assay (Clift et al., 2017) could aid the understanding of PGRMC1 function in future studies.

The uncertain function of Progesterone Receptor Membra Component 1 in the early embryo

Limited functional data on the role of PGRMC1 during early mammalian embryogenesis are available in the literature. Both the PGRMC1 transcript and protein are expressed in the bovine

The unknown function of Progesterone Receptor Membra Component 1 in the oviduct

The oviduct is the anatomical site where fertilisation and early embryonic development occur. As such, oviductal cells concur to provide the physiological environment for these essential develop-
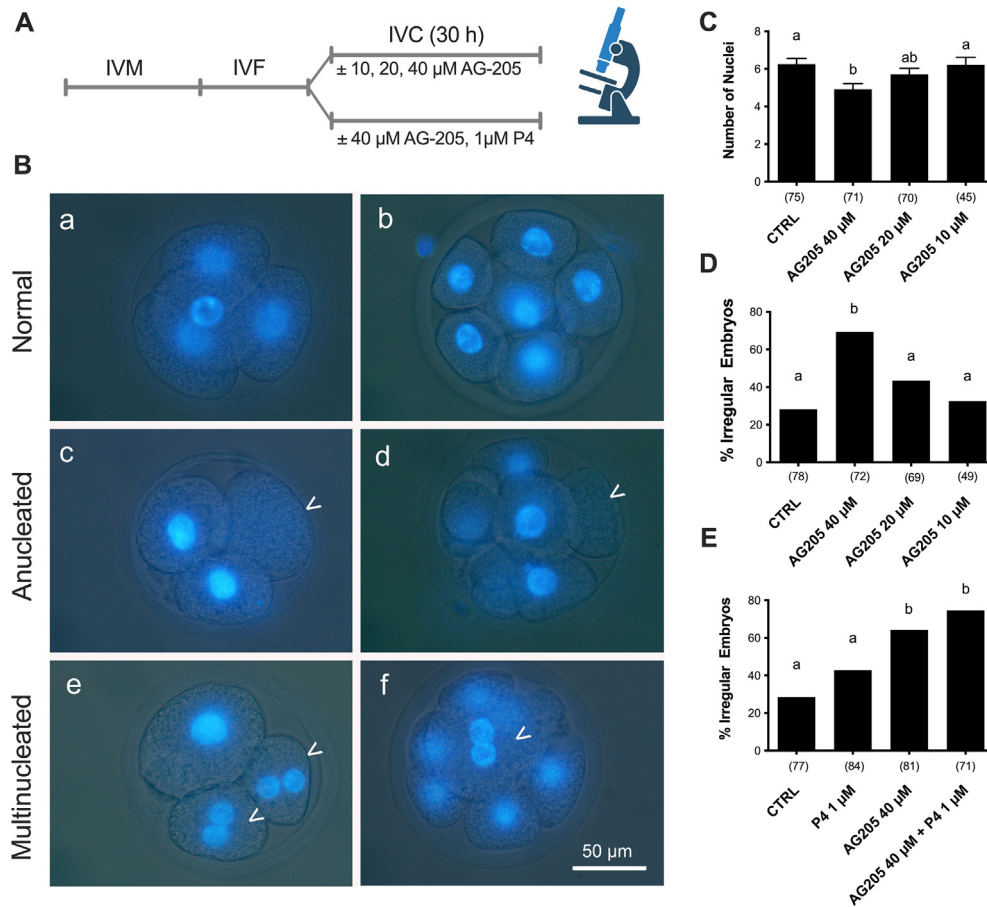
Fig. 2. Effect of AG-205 treatment on bovine embryo cleavage. Bovine zygotes were obtained in vitro from fully grown immature oocytes according to the standard protocol for in vitro embryo production in use in our laboratory (Lodde et al., 2021; Garcia Barros et al., 2023). After In Vitro Fertilisation (IVF), presumptive zygotes were cultured for 30 hours at 38 °C in 5%C02, 5%O2 in humified air in serum-free Synthetic Oviductal Fluid (SOF) medium supplemented with 8 gr/L Bovine serum Albumin, Fatty Acid-free, and 1.5 mM glucose and treated with 0, 10, 20 or 40 μM AG-205 dissolved in Dimethyl sulfoxide (DMSO). In a second set of experiments, zygotes were treated under control conditions, in the presence of 1 μM Progesterone (P4), 40 μM of AG-205, or a combination of the two. After culture embryos were fixed, mounted in a mounting medium with the nuclear stain 2-(4-Amidinophenyl)-6-indolecarbamidine (DAPI) and analyzed by bright field and epifluorescence microscopy. The number of blastomeres and the number of DAPI stained nuclei were counted. Embryos were classified as "Normal" when all the blastomeres were mononucleated, while they were classified as "Irregular" when one or more blastomeres were multinucleated or anucleated. Differences between the number of Normal and Irregular embryos were analysed by Chi-square Fisher exact Test, while differences in the number of nuclei per embryo were analyzed by non-parametric Kruscall-Wallis tests followed by Dunn's test using Graphpad Prism v.8. Values of $P < 0.05$ were considered statistically significant. Data shown in the graphs were obtained in four biological replicates; a number of embryos analyzed are shown in brackets. These data were presented at the 51st Annual Meeting of the Society for the Study of Reproduction (2018), New Orleans, Louisiana (USA), at the 19th International Congress on Animal Reproduction (2022) Bologna (ITALY) and re-elaborated in Giovanardi and Lodde (2021) Illustration in (A) shows the experimental design. Images in (B) are representative of normal, anucleated and multinucleated blastomeres. Graph in (C) shows the mean number (±SEM) of DAPI-stained nuclei in embryos treated with increasing concentration of AG-205. Graph in (D) shows the percentages of normal and irregular embryos treated with increasing concentration of AG-205. Graph in (E) shows the percentages of normal and irregular embryos treated with 1 μM P4, 40 μM AG-205, or a combination of the two. a, b: $P < 0.05$.

mental processes. PGRMC1 is highly expressed in oviductal cells as revealed by immunohistochemical analysis (Luciano et al., 2011; Saint-Dizier et al., 2012). Nevertheless, to the best of our knowledge, functional studies have not been yet conducted in any mammalian species. Therefore, the role of PGRMC1 in controlling oviduct function and indirectly concurring with embryonic development is currently unknown.

Concluding remarks

The data reviewed in this paper support the hypothesis that PGRMC1 participates in oocyte acquisition of developmental competence both extrinsically, through the regulation of follicular growth and intrinsically, participating in chromosome segregation and polar body emission during oocyte maturation. While in granulosa cells, much experimental evidence supports the idea that PGRMC1 and its binding partners mediate some of the noncanonical action of P4, whether the function during oocyte maturation is controlled by P4 has not been tested.

Mechanistically, intense research is needed to reveal all binding partners, sites of action and the role of post-translational modifications. However, the mechanisms by which PGRMC1 regulates granulosa cell division and oocyte maturation seem to be conserved. Even though data suggest that some of these functions are conserved in the early embryo, this hypothesis still needs to be confirmed, because this assumption is based on the use of an inhibitor, AG-205, which may also alter PGRMC1-independent processes.

Finally, since PGRMC1 is expressed in many other organs and cell types of the reproductive system that are ultimately responsible for oocyte quality, it is expected that PGRMC1 has additional functions that still must be discovered. We hope that the discussion presented in this paper will encourage future research in this fascinating field.

Ethics approval

Not applicable.

Data and model availability statement

Data or models were not deposited in an official repository. No new datasets were created.

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V.L, A.M.L. and **F.F.** designed and wrote the manuscript. **V.L, A. M.L, R.G.B, G.S, G.G, F.F.** performed experiment presented in the manuscript. All authors have approved the final version of the manuscript.

Declaration of interest

The authors declare no conflict of interest

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