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Nurturing the egg: the essential connection between cumulus cells and the oocyte

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ABSTRACT

The determinants of oocyte quality remain uncertain. Under suitable conditions, which have yet to be defined, the gamete grows and acquires the competence to resume meiosis, be fertilised and undergo embryonic development at least beyond genome activation, after which the blastomere is autonomous enough to adapt to the specificity of its environment. This review describes the central role played by the oocyte in reproductive success and how communication between cumulus cells and the oocyte are essential to proper oogenesis and the quality of the resulting gamete. While most attempts to improve oocyte quality have been directed at gonadotrophin-based systemic endocrine signalling, it is proposed that parallel control of fertility may act locally within ovarian follicles through intimate cooperation between somatic cells and the oocyte *via* the network of transzonal projections. This intercellular communication may prove to be more sensitive to environmental conditions than systemic endocrine signalling, which is essential for many non-reproductive tissues.

Keywords: cell signalling, developmental competence, follicle, folliculogenesis, intercellular communications, oocyte, oogenesis, ovary, transzonal projections.

Introduction

Oogenesis and folliculogenesis are two processes that need to be studied to improve scientific understanding of the physiological mechanisms that ensure the production of fully functional eggs. Both processes include stages that are pivotal for female fertility and for the success of livestock herd improvement efforts based on transfer of embryos produced *in vitro*. The conditions required for the acquisition of developmental competence by oocytes maturing in the follicle or *in vitro* are still unclear and under investigation.

Intercellular communication

An ovarian follicle is an assembly of communicating interdependent cells that supports the development of the gamete inside. Whereas the development of the antral follicle is controlled remotely to a large degree through the hypothalamic–pituitary–gonadal (HPG) axis, follicular maturation and oogenesis depend more on intra-follicular communication. Folliculogenesis refers to the entire process by which the follicle grows to become fully functional. The natural ovarian cycle involves growth stimulation by follicle stimulating hormone (FSH) with negative feedback *via* oestradiol secretion by granulosa cells (Liu and Hsueh 1986). Interestingly, no signalling pathway external to the ovary has been found to act directly on the oocyte. In fact, the oocyte has no receptors for FSH or luteinising hormone (Calder *et al.* 2005) and even secretes repressors of gonadotrophin receptor expression in adjacent cells (Eppig *et al.* 1997). It therefore must receive its external signals *via* chains of intermediary cells. For example, the receptor tyrosine kinase KIT is expressed on the oocyte and on theca cells whereas the KIT ligand is secreted by granulosa cells and influences oocyte growth and theca cell differentiation (Reynaud *et al.* 2001).

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During the pre-ovulatory period, the LH surge is detected by theca and granulosa cells and transmitted *via* the epidermal growth factor signalling pathway as epiregulin, amphiregulin, betacellulin, neuregulins and other secretions (Conti *et al.* 2006) to cumulus cells, which respond by halting the flow of cyclic AMP to the oocyte, thus triggering resumption of meiosis in the gamete (Santiquet *et al.* 2013; Zeng *et al.* 2013; Gilchrist *et al.* 2016). This flow of cAMP is not endocrine but occurs rather through tubular channels that extend from the cumulus cells through the zona pellucida and make direct contact with the oolemma (Santiquet *et al.* 2013; Gilchrist *et al.* 2016). These channels are called transzonal projections, and the area of contact between them and the oocyte is not a simple hole but contains active pores called gap junctions.

Meanwhile, the oocyte ensures the maintenance of follicular growth. Its presence is sensed by granulosa and theca cells through secreted factors such as growth-differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) (Su *et al.* 2004; Sugiura *et al.* 2010). These intercellular communications orchestrate the growth, the differentiation, and the demise of the follicle. The gamete is thus in an essential partnership with its surrounding somatic cells.

Timescale of folliculogenesis and oogenesis

This intimate partnership is established and maintained throughout folliculogenesis. The timescales that apply to ovarian function are unusual. The capacity to remain in a latent state for decades makes the ovarian follicle in large mammals a highly unique structure. Time is similarly discontinuous for the oocyte, which awaits follicular recruitment to initiate intense developmental activity, during which it grows to its full size only to return to a new phase of latency and await fertilisation. Although studies tend to focus on the final phase of follicular growth, namely the 20 days or so during which late antral follicles respond to FSH, the process of recruitment from reserves and pre-antral growth is considerably longer. One of the more comprehensive descriptions of follicular ontogeny in large mammals is that of the human case, in which the initiation and basal growth phases span more than 6 months (Gougeon 1996) (Table 1). It should be kept in mind that in large mammals, follicles reaching the final pre-ovulatory phase have been exposed to the endocrinological cycles of several previous follicular waves.

Is FSH needed early?

This exposure to circulating gonadotrophins (primarily FSH) has been the focus of conflicting reports. In many cases, repeated ovarian stimulation does not seem to have any major effect on the number and quality of recovered oocytes or eggs (Caligara *et al.* 2001; Sood *et al.* 2017; Paul *et al.* 2019; Tutt *et al.* 2021). The number of follicles recruited per wave in a given animal is very repeatable over time

Table 1. Evolution of bovine folliculogenesis across developmental stages.

Characteristics	Follicle developmental stage						
	Primordial	Primary	Secondary	Early antral	Medium antral	Large antral	Preovulatory
Timeline	Years/decades[a]	120 days[a]	65 days[a]			20 days[a]	
Follicular size	≤40 μm[b]	40–130 μm[b]	250 μm[b]	500 μm[b]	3–6 mm	≥8 mm	15–20 mm
Oocyte size	30 μm[b]	30 μm[b]	50–70 μm[b]	90–100 μm[b,c]	120–130 μm[c]	120–130 μm	120–130 μm
Zona pellucida	None[c]	Sparse[c]	Few[c]	All[c]	All[c]	All	All
Presence of TZPs	–	–	+	++	+++	+++	+++
Chromatine compaction	Partial[c]	Partial[c]	Partial[c]	Partial[c] >80% GV0[d]	Partial GV1 = 25%; GV2 = 50%, Fully compacted GV3 = 25%[e]	?	?
Transcriptional activity in oocyte nucleus	0%[f]	12%[f]	66%[f]	100%[f]	Highly repressed in GV1–GV2 and silenced in GV3[g]	?	?
Capacity to resume meiosis and reach MII	0%	0%	0%	20%[h]	81%[h]	≥90%	≥90%
Embryonic developmental competence	0%	0%	0%	0%	30–40%[i]	65%[i]	≥80%

Note: Superscript letters indicate references: [a]Gougeon (1996); [b]Braw-Tal and Yossefi (1997); [c]Fair *et al.* (1997*b*); [d]Lodde *et al.* (2007); [e]Dieci *et al.* (2016); [f]Fair *et al.* (1997*a*); [g]Lodde *et al.* (2008); [h]Fair *et al.* (1995); [i]Lonergan *et al.* (1994).

(Burns *et al*. 2005) and is heritable (Bényei *et al*. 2004). These observations suggest that pre-antral growth is genetically driven and largely unresponsive to external cues including fluctuations in circulating gonadotrophins and steroid hormones. Murine studies confirm this. When cumulus–oocyte complexes from pre-antral follicles in Day 12 animals (in which the ovaries do not contain antral follicles and the oocytes are known to be incapable of resuming meiosis) were cultured for 10 days (in a medium free of FSH but containing fetal calf serum) then fertilised and transferred, live pups were born (Eppig and Schroeder 1989). This suggested that the presence of theca and granulosa cells or FSH was not essential. Using gene inactivation models, it was then shown that in the absence of gonadotrophin receptors, pre-antral folliculogenesis can be sustained but stops at the secondary stage before antral formation (Kumar *et al*. 1997; Dierich *et al*. 1998), strengthening the hypothesis that only antral follicles benefit from gonadotrophins.

However, a different picture emerges from *in vitro* culture of ovarian cortex and follicles. FSH even at low concentrations has been shown to promote pre-antral growth in several species (Demeestere *et al*. 2005; Kreeger *et al*. 2005; Wang *et al*. 2011). Its presence may not be essential, but its effect on the pre-antral follicle is undeniable. In addition, coupling of cumulus cells with the oocyte through transzonal projections fails to occur in the absence of FSH or GDF9 (Albertini *et al*. 2001). In the experiment with ovarian follicles from day 12 mice, the surface on which the cumulus–oocyte complexes were deposited had to be coated with collagen to prevent cumulus cell migration and to maintain the somatic–gamete interconnectivity, without which oocyte growth and acquisition of competence was compromised (Eppig and Schroeder 1989). These observations suggest that pre-antral development is robust and may progress with minimal support, but that endocrine stimulation and cumulus cell–oocyte physical connectivity are essential for subsequent developmental stages. Since transzonal projections begin to form during the secondary follicle stage (Table 1), pre-antral conditions may have little impact on the endocrine support required at the antral stage but may affect the quality of the transzonal network. In this sense, pre-antral development might not be entirely preprogrammed and could contribute to follicular outcome.

Although mouse oocytes of good quality are obtained in high yield from follicular culture, it is proving to be more difficult to achieve the same success in the case of large mammals (McLaughlin and Telfer 2010). Adequate *in vitro* support for the early stages of folliculogenesis (e.g. primary or secondary antral) has been designed for cattle, pigs and humans (Pangas *et al*. 2003; Xiao *et al*. 2017). The best system so far involves 3D culture of follicles encapsulated sequentially in matrices of different density to mimic the *in vivo* environment at each developmental stage (Pangas *et al*. 2003; Kreeger *et al*. 2005; Xiao *et al*. 2017). Human follicles thus cultured from the secondary pre-antral stage have brought gametes to full size with the ability to resume meiosis (Xiao *et al*. 2015). A more sophisticated system involves ectopic culture of up to five tissues (follicle/ovary, Fallopian tube, endometrium, cervix and liver) interconnected through microfluid channels allowing the exchange of metabolites and enabling precise stimulation through exogenous administration (pulse) and wash-off (chase) of hormones, drugs and so on (Xiao *et al*. 2017). A common observation is that flat culture often leads to poor communication between cumulus cells and the oocyte and ultimately to follicular demise (Eppig and Schroeder 1989; Xiao *et al*. 2015, 2017). Moreover, even when oocytes reach full-size in such cultures, they often have a limited transzonal network and are meiotically incompetent (Telfer and Andersen 2021). These reports all suggest that physical connection to the cumulus complex *via* a fully functional network of tubular channels is crucial for the developmental competence of the oocyte.

The transzonal projection network

Oocytes denuded even at late stages of oogenesis are considerably less likely to resume meiosis (Macaulay *et al*. 2016). Denuded oocytes can sometimes be salvaged by co-culture with fully-enclosed cumulus–oocyte complexes (Luciano *et al*. 2005; Scantland *et al*. 2014). It has also been noted that oocytes matured in groups often fare better than those matured individually (O'Doherty *et al*. 1997). We note that co-cultured cumulus cells are difficult to separate from an oocyte matured *in vitro* because their expansion tends to surround the previously denuded gamete. Cumulus cells detaching from one oocyte and reattaching to a neighboring denuded oocyte has been observed (Fig. 1). It is not known

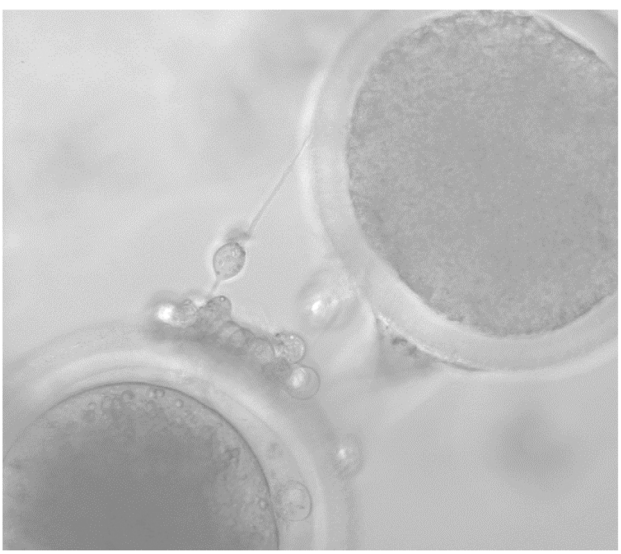

Fig. 1. Bovine cumulus cell (bottom left) extending a cellular projection towards the denuded GV-stage oocyte (upper right). Photo taken by Dr Angus Macaulay.

if such new connections are functional and contribute significantly to supporting the maturation of the oocyte, but the above observations suggest collectively that cumulus cells help with the maturation of oocytes in proximity, by secreting what are likely labile factors.

The major cytological and physiological developments that occur during folliculogenesis in cattle are summarised in Table 1. Little doubt remains that the intimate physical contact between the oocyte and its surrounding somatic cells lasts throughout the process. As the follicle progresses through its initial growth phase, the number of pre-granulosa cells surrounding the oocyte is limited but definitely increases, especially those in close contact with the gamete (Braw-Tal and Yossefi 1997). Pre-granulosa cell cytoplasmic membranes are initially juxtaposed and attached to the oocyte oolemma *via* the zonula adherens and desmosome structures (Motta *et al.* 1994; Fair *et al.* 1997*a*, 1997*b*). Following recruitment, the follicle and its oocyte grow conjointly, the surrounding somatic cells dividing to maintain complete coverage of the gamete surface (Braw-Tal and Yossefi 1997). The initial growth phases are slow, requiring months to complete (Gougeon 1996). Transzonal projections are established as the zona pellucida is secreted, which occurs at the end of the secondary stage of folliculogenesis (Fair *et al.* 1997*b*). This marks the onset of a phase during which cells close to the gamete differentiate into cumulus cells while those farther away remain granulosa cells. In mice, the glycoprotein shell is secreted solely by the oocyte (Philpott *et al.* 1987) whereas in large mammals, cumulus cells also secrete zona pellucida proteins (Sinowatz *et al.* 2001; Kölle *et al.* 2007). The ultrastructure of transzonal projections has been investigated thoroughly, beginning with electron microscopic observations in the mid 1960s (Hertig and Adams 1967). Most of our current understanding of these projections comes from the work of Professor David Albertini who pioneered the study of transzonal projection dynamics and interconnectivity between the somatic cells and the oocyte in relation to folliculogenesis and oocyte quality (Anderson and Albertini 1976; Carabatsos *et al.* 1998; Albertini *et al.* 2001; Combelles *et al.* 2004; Plancha *et al.* 2005; Li and Albertini 2013). It was also shown during this time that the number of transzonal projections in human oocyte complexes evolves during folliculogenesis (Motta *et al.* 1994). The cellular extensions are not static structures but evolve within the growing follicle. At least two types can be distinguished based on cytoskeleton backbone. Most transzonal projections have an actin filament core while some are made of microtubules (Can and Albertini 1997; Albertini *et al.* 2001; El-Hayek *et al.* 2018). Networks of transzonal projections have been described in many mammalian species. Fig. 2 shows those of pig, cattle, and mouse oocytes. Species differences include thinner and less straight networks in mice (Baena and Terasaki 2019). Network development is similar in humans, pigs and cattle (Coticchio *et al.* 2014).

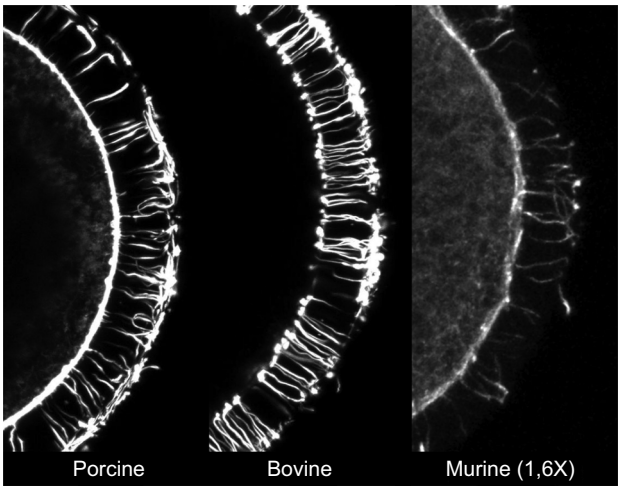

Fig. 2. Comparison of the network of transzonal projections in three species. Actin filaments are stained with phalloidin. The mouse oocyte image is magnified 1.6 times relative to the others. Photos taken by Alexandre Bastien.

The network is essential for folliculogenesis, one of its main known roles being to shuttle cyclic nucleotides to the oocyte cytoplasm to maintain meiotic arrest (Gilchrist *et al.* 2016). Transzonal projections are not open-ended. Molecules are transferred through them *via* gap junctions, specialised pores that span both membranes (Kidder and Mhawi 2002; Luciano *et al.* 2011). The LH surge detected by theca and granulosa cells is conveyed to cumulus cells through paracrine signalling, which leads to destabilisation, closure and cessation of molecular transfers *via* gap junctions (Santiquet *et al.* 2013; Gilchrist *et al.* 2016). The cumulus cells thus constitute the final layer of control over the interruption of meiosis. Another essential role played by the same mode of transfer is the supply of energy substrates to the oocyte (Sutton-McDowall *et al.* 2010). The oocyte is fully dependent on this exogenous delivery of lactate, pyruvate and creatine to produce ATP (Rieger and Loskutoff 1994). It is endowed with a very peculiar contingent of mitochondria estimated to number from 30 000 to 1 000 000 (Van Blerkom 2011) but individually very inefficient at producing ATP by glycolysis or oxidative phosphorylation (Rieger *et al.* 1992; Rieger and Loskutoff 1994). In many species, including large mammals, oocyte mitochondria undergo conformational transformations during folliculogenesis, passing from small and round to a hooded immature form that progresses to the familiar post-fertilisation oval shape once embryonic genome activation is complete (Van Blerkom 2011). The purpose of this transformation remains unclear. Nevertheless, up to the transzonal projection withdrawal that occurs following meiosis resumption, cumulus cells control the availability of ATP to the oocyte. This is important, since the gamete needs much energy to manage and transcribe its genome to constitute the maternal reserves of stored mRNA and to translate the proteins that will

be needed during the upcoming period of transcriptional silence (Fair *et al.* 1995; Lodde *et al.* 2008) (Table 1).

The physiological support provided by cumulus cells through transzonal projections was long thought to be limited to small molecules (≤1 kDa) capable of passing through gap junctions. However, larger cargos such as mRNA granules and lipids have been shown to pass from cumulus cells to the oocyte (Macaulay *et al.* 2014, 2016). These transfers are believed to occur *via* vesicles secreted at the tip of the projections (Macaulay *et al.* 2014, 2016). The contact between cumulus cells and the oocyte thus resembles neuronal synapses both structurally and functionally (Allworth and Albertini 1993; Macaulay *et al.* 2014). And just as neurones do not synthesise each protein in the cell body and transport it over several millimetres or a metre of axon but instead package mRNA into ribonucleoparticles sometimes with ribosomes attached and transport these for local translation (El Fatimy *et al.* 2016; Muzio and Cascella 2021), so do cumulus cells arrange for remote translation of proteins used in the oocyte. This has been shown by transfecting cumulus cells with a non-endogenous gene (FMRP-GFP) and finding the mRNA in the oocyte cytoplasm (Macaulay *et al.* 2014, 2016). It is not yet clear if such transcripts are translated in the oocyte cytoplasm or in the transzonal projection bulging ends followed by transfer of the proteins. Both mechanisms are possible and not mutually exclusive.

Other intercellular transfers of large molecules

Direct transfer of large molecules between two cells has been described for many cell types, such as neurones, immune cells and epithelial cells (Domhan *et al.* 2011; Nawaz and Fatima 2017). It is often mediated through tunnelling nanotubes (TNTs), which are actin-based cytoplasmic projections that can stretch from 50 nm in length to 200 nm (Nawaz and Fatima 2017). Unlike transzonal projections, TNTs are open ended and thus allow bidirectional transfers of material. They have been shown to deliver small molecules such as ions and metabolites but also lipid droplets, lysosomes, viral genomes, entire viruses and even mitochondria (Nawaz and Fatima 2017). Even though cumulus cell transzonal projections are wider than TNTs, it is not known if they are as permissive. Mitochondria do fit inside them and have been found throughout their length (Albertini *et al.* 2001). However, mitochondrial transfer from cumulus cell to the oocyte has not been documented although proof of such transfer could be evident as mitochondria from both cells do not have the same shape.

The fully developed bovine transzonal network is composed of several thousands of channels. Since the oocyte is the founder cell of the individual animal, transfer of any matter able to destabilise or integrate into the oocyte genome is potentially harmful. Compared to modifying the oocyte genome, modifying a somatic cell genome carries no risk for posterity. On the other hand, this could be a source of random genomic recombination and hence new alleles that provide competitive advantages in specific environments. For example, genomic reorganisation is a major factor in resistance to infectious disease (Rubelt *et al.* 2017). To put into perspective this risk of transferring potentially genome-destabilising exogenous DNA, the average mutation rate per nucleotide per generation is estimated at $<10^{-7}$ for a typical large mammalian species (human, cattle) having a genome length of about 3 billion base pairs (Lynch *et al.* 2016). This low intergenerational mutation rate suggests the presence of a controlled transfer rather than a transfer of random molecules. In addition to supplying the oocyte with essential molecules, cumulus cells likely play a protective role by sequestering harmful compounds absorbed from the environment. They have been shown to internalise many types of molecules including follicular fluid lipids, secreted vesicles and even bacterial particles (Hernandez-Gonzalez *et al.* 2006; Richards 2007; del Collado *et al.* 2017). Absorbed material is not transferred spontaneously to the oocyte but seems instead to be the object of intracytoplasmic sorting and possibly repackaging or degradation.

Transzonal projections and oocyte developmental competence

Transzonal projections apparently do not deliver ribonucleoprotein particles constantly, at least not mRNA, before or after the LH surge or during spontaneous meiosis resumption when the cumulus–oocyte complex is removed from the follicle. In cattle ovaries incubated after slaughter, transzonal projections load up on RNA in conjunction with acquisition of oocyte developmental competence (Macaulay *et al.* 2014, 2016). It has been shown that oocytes aspirated from ovaries quickly after slaughter have limited developmental potential (Blondin *et al.* 1997). Incubating in saline for 4 h before aspiration more than doubled the percentage of embryos that reached the blastocyst stage after *in vitro* oocyte maturation, fertilisation and embryo development (Blondin *et al.* 1997). It is unclear why this difference is so large, since the oocyte remains in meiotic arrest while inside the ovarian follicle. It has been shown that RNA accumulates inside transzonal projections during this time (Macaulay *et al.* 2014, 2016). Transfer of RNA to the oocyte could be part of the final phase of differentiation leading to the acquisition of developmental competence. This would be consistent with the observation that oocytes in growing follicles are less developmentally competent than those in early atretic follicles (Blondin and Sirard 1995). Oocytes salvaged after slaughter have better developmental potential when surrounded by cumulus complex displaying early signs

of expansion than when the complex is very thin or very expanded (Blondin and Sirard 1995). This led to the theory that early atresia is beneficial for the acquisition of developmental potential (Blondin and Sirard 1995; Blondin *et al.* 1997, 2002; Nivet *et al.* 2012). The time window of this beneficial effect associated with early demise is rapid and spans only a few hours before an irreversible decline in quality. *In vivo* uncoupling from the follicular environment occurs after the LH surge, when theca and granulosa cells begin differentiation to become a fully functional corpus luteum while cumulus cells close their gap junctions to cut the flow of cAMP to the oocyte to allow meiosis to resume (Santiquet *et al.* 2013). Disconnection of the transzonal projections has been shown to involve the EGF signalling pathway (Abbassi *et al.* 2021). A mild induction of transformation can be induced during follicular growth by withdrawal of FSH. These 'coasting' protocols in which oocytes are recovered 48–54 h after FSH injection have been shown to result in very robust developmental competence in Holstein cows (Blondin *et al.* 2002; Nivet *et al.* 2012). It is not known if post-FSH timing coincides with RNA loading of transzonal projections. Material transfer during late folliculogenesis is known to occur in *Drosophila*, in which nurse cells (equivalent to cumulus cells) go through a process called 'cytoplasmic dumping' of all their contents into the oocyte, except for the nucleus, which is retained by a mesh of actin filaments (Guild *et al.* 1997; Spracklen and Tootle 2013). This is an extreme example of cellular devotion to nurturing the egg cell entrusted with the future of the species.

Why so much focus on oocyte quality?

While continuity of physiological support from conception to birth is essential for the delivery of healthy offspring, the key determinants of fertility appear to be developmental competence and a receptive uterine environment. Once gestation is underway, the investment of material resources is already considerable, and halting it would be costly. Based on *in vitro* production of cattle embryos as a proxy for the pivotal steps, oocyte quality seems to be a crucial component of reproductive success (Fig. 3).

Most protocols of *in vitro* production of cattle embryos start with oocytes at the germinal vesicle stage, making a maturation step necessary prior to fertilisation. Most embryo failures occur very early in development, prior to genome activation (Fig. 3). Once this critical step is passed, failure drops to 5–10% during the transition to the blastocyst stage. Of all oocytes fertilised in a typical embryo production run, about 40–50% are viable at transfer (Holm and Callesen 1998; Numabe *et al.* 2000; van Wagtendonk-de Leeuw *et al.* 2000; Hansen 2020) and about 18–32% of these fail before or shortly after implantation/attachment, whereas late gestational arrests are far fewer (Romano *et al.* 2016). These very approximate figures reveal

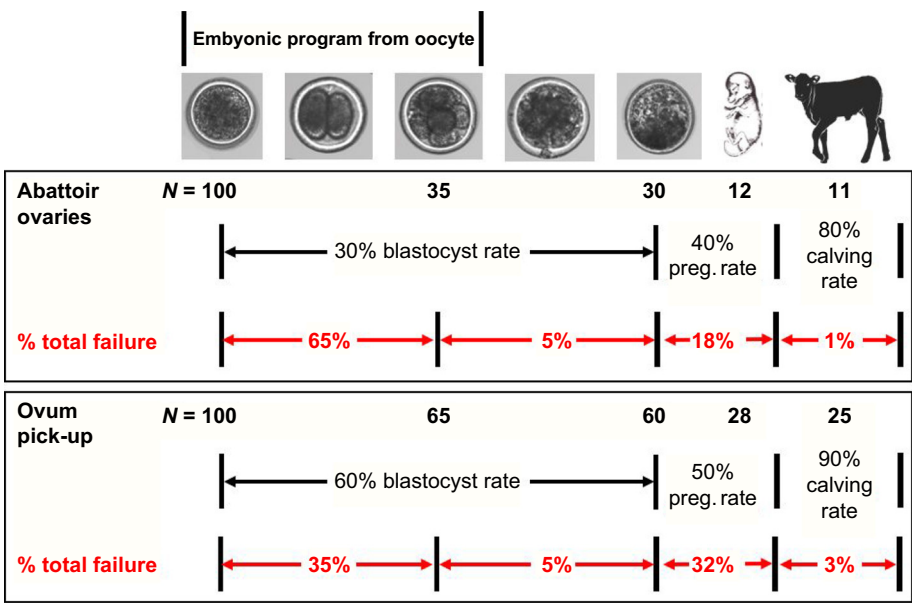

Fig. 3. Rates of developmental failure from periconception through birth for *in vitro* cattle embryo production. Numbers are rounded estimates contrasting oocytes collected from abattoir ovaries or by ovum pick-up from live stimulated animals. The total percent failure is based on standard success rates following *in vitro* maturation, fertilisation and development. Post-transfer failure rates are based on published survival rates (Holm and Callesen 1998; Hansen 2020). Photos of oocytes and embryos are by Isabelle Laflamme. Foetus and calf drawings are by Dr Romanes, G.J. (1892) and Pixabay, respectively.

the impact of oocyte quality on the success of embryos produced *in vitro*. They indicate that most oocytes sent down the standard *in vitro* pipeline do not generate a live calf, and that 35–65% of all losses occur very early, while the embryo is still under the control of the maternal program. The importance of the last phase of folliculogenesis is revealed by contrasting the developmental competence of oocytes matured *in vitro* to those matured *in vivo* (Table 2). Despite confounding factors that could explain some of the observed variance, *in vitro* maturation clearly does not replicate very adequately this last phase of oocyte preparation. This is true even in mice, despite the relative success of *in vitro* oogenesis and folliculogenesis with minimal complexity (Eppig and Schroeder 1989).

Two aspects determining the effectiveness of transzonal projections to consider are (1) how the network is established and maintained and (2) network functionality in terms of transferring material to the oocyte. The underlying processes could be distinguished based on whether poor follicular support or incomplete follicular and oocyte differentiation has caused developmental incompetence. It has been shown that networks are less developed in women who have long reproductive histories (El-Hayek *et al.* 2018). In the case of porcine oocytes, the actin filaments are more easily destabilised by heat stress (Yin *et al.* 2020). Lower competence of bovine oocytes has also been associated with less developed transzonal networks, and oocytes matured in follicular culture under suboptimal conditions frequently display poor connectivity with cumulus cells as well as turning out to be developmentally incompetent (Modina *et al.* 2007).

Modulating fertility during suboptimal periods

The key steps of folliculogenesis and oogenesis amount to progressive acquisition of maternal reserves that enable the oocyte to resume meiosis. Meiotic capacity is re-acquired before the acquisition of developmental competence, which requires complete shutdown of transcription in conjunction with genome compaction (Table 1). This stepwise progression could allow biological quality control since the follicle and the oocyte must stay synchronous through bidirectional communication. It could also be a modulator of fertility at any point from early folliculogenesis up to the final phase of post-LH preparation.

The need for this multi-step control over oocyte quality is unclear. Many species are known to suffer from reduced fertility periodically and sometimes chronically. Dairy cattle and pigs exhibit lower fertility during periods of heat stress (Graves *et al.* 2018; Hansen 2020). Although essential for the perpetuation of a species, fertility is expendable for the individual facing a threat to its own survival. Since bearing offspring is such a demanding process for female mammals, it can be a perilous project when conditions are unfavourable. This may explain why reproductive performance is so sensitive to environmental conditions and the metabolic state of the animal. Modulating fertility in accordance with resource availability and body condition is thus believed to be an adaptative trait to improve fitness to maximise the survivability of the progeny (Lawson and Borgerhoff Mulder 2016). This adaptable physiology makes regulation of the reproductive system highly complex. Whereas complete shutdown would hinder secretion of gonadal steroid hormones that have beneficial systemic effects elsewhere, sustaining endocrine secretion while lowering fertility necessarily requires a wide array of metabolic sensing signalling pathways to act in parallel to the canonical endocrine signalling that drives ovarian functions and uterine receptivity.

Transcriptomic surveys and gene expression modelling have shown that follicular cells express in a stage-dependent manner many metabolic pathways including lipid oxidation, which is more active during the transition to the antral follicle (Bunel *et al.* 2014; Peñalver Bernabé *et al.* 2019). Such metabolic flexibility throughout follicular development could support the modulation of fertility in response to environmental conditions.

Conclusion

The intimate relationship between the oocyte and the surrounding cumulus cells is essential to the development of

Table 2. Blastocyst yield *in vitro* from oocytes matured *in vitro* (IVM) or *in vivo* (IVF).

Species		% MII	% Blastocysts	Confounders	References
Mouse	IVM	100	50–60	Genetic strain	Santiquet *et al.* (2017)
	IVF	N/A	80–85		Truong and Gardner (2017)
Bovine	IVM	82	30–40	Follicle size	Plourde *et al.* (2012)
			60–80 (OPU)		Nivet *et al.* (2012)
	IVF	N/A	65–75		Rizos *et al.* (2002)
Human	IVM	30–65	5–30	PCOS	Ellenbogen *et al.* (2014)
	IVF-ICSI	N/A	40–55	Maternal age	Stimpfel *et al.* (2019)

a high-quality oocyte capable of resuming meiosis, being fertilised and undergoing embryonic development at least to the blastocyst stage. This connectivity is ensured by the network of transzonal projections. *In vitro* culture of ovarian follicles under suboptimal conditions has shown that resulting full-size oocytes are incompetent, a consequence of just getting by with an underdeveloped network of transzonal projections. The formation of an extensive and fully functional network is therefore a strong predictor of a good oocyte quality.

Premature completion or partial loss of connectivity with cumulus cells will prevent the oocyte from becoming developmentally competent. Full-sized oocytes acquiring developmental potential through a late maturation step while transzonal projections are still actively shuttling RNA has been reported. The transzonal projection network must therefore be not only extensive but fully functional.

This implies two separate but related aspects of folliculogenesis and oogenesis having a direct impact on oocyte quality, either early through modulation of network development or later through transfers of material *via* the transzonal projections. This could have biological relevance by adjusting fertility when environmental conditions are suboptimal (food less accessible, heat stress, etc.) without completely shutting down systemic endocrine signalling. This relationship needs to be studied in greater depth.

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