INTRODUCTION

Oocyte maturation is a physiological process that precedes and is essential for successful fertilization and subsequent embryonic development (Lonergan and Fair, 2016). This process is initiated a few days prior to ovulation and involves progressive changes in ovarian follicles. These include preantral to early antral transition (Palma et al., 2012) and eventual acquisition of competence to resume meiosis and fertilizability (Paulini et al., 2014). Oocyte maturation is characterized by both nuclear and cytoplasmic maturation. The former is a complicated process that includes the resumption of meiosis post luteinizing hormone (LH) surge or oocyte release from a follicle (Luciano and Sirard, 2018). Mammalian oocytes enter the early stages of meiosis during fetal life but remain arrested at prophase I until they become committed to ovulation in response to an LH surge (Grøndahl, 2008). Meiotic resumption induced by the LH surge is further stalled at metaphase II (Lonergan and Fair, 2016) which is then completed following fertilization (Grøndahl, 2008). In contrast, cytoplasmic maturation is associated with organelle reorganization and storage of mRNAs, proteins, and transcription factors that are requisites for early embryonic development (Ferreira et al., 2009).

In vitro maturation (IVM) of oocytes is the procedure where the immature oocytes are cultivated in a laboratory until they are mature. Since IVM oocytes generally have low developmental competence as compared to those matured in vivo, development of an optimal IVM culture system by fine-tuning culture conditions is crucial to maintain high quality. In-depth knowledge and a deep understanding of the in vivo physiology of oocyte maturation are pre-requisites to accomplish this. Within ovarian follicles, various signaling pathways that drive oocyte development and maturation regulate interaction between oocytes and surrounding somatic cells. This review discusses the sonic hedgehog (SHH) signaling pathway, which has been demonstrated to be intimately involved in folliculogenesis and oocyte maturation. Advances in elucidating the role of the SHH signaling pathway in oocyte maturation will aid attempts to improve the current inferior in vitro oocyte maturation system.

Keywords: in vitro oocyte maturation, ovarian follicle, sonic hedgehog signaling
Oocytes can be completed, cytoplasmic maturation usually remains incomplete, thereby possibly leading to poor embryonic development (Marchal et al., 2003). Since the nuclear maturation of oocytes must be accompanied by a variety of events that occur within the cytoplasm to complete embryonic development (Krisher, 2004), various attempts have been made to improve developmental competence of IVM oocytes in different species, including pigs (Appeltant et al., 2016; Jeon et al., 2020), cattle (Abd El-Aziz et al., 2016), and camels (Moawad et al., 2020; Saadeldin and Cho, 2021). These include supplementation of porcine IVM medium with antioxidants such as resveratrol (Lee et al., 2015) and spermine (Jin et al., 2016) to scavenge reactive oxygen species produced during the IVM process. In addition, attempts to mimic the intrafollicular environment have involved the addition of precursors of cholesterol synthesis, such as lanosterol (Lee et al., 2016) and follicular fluid meiosis-activating sterol (FF-MAS) (Marín Bivens et al., 2004) to porcine or mouse IVM medium. Furthermore, pre-IVM incubation with dibutyryl cAMP (dbcAMP; the cAMP analog) and 3-isobutyl-1-methylxanthine (IBMX, a phosphodiesterase inhibitor) improved bovine IVM by elevating cAMP levels (Sugimura et al., 2018). However, despite these efforts, IVM oocytes continue to possess lower developmental competence as compared to those matured in vivo. Elucidation of the mechanisms involved in oocyte developmental competence is therefore essential to develop strategies for further improvement of IVM efficacy. This in turn requires a better understanding of the in vivo physiology of oocyte maturation in order to be able to optimize IVM culture conditions for better performance.

The functional unit of the ovary i.e. the ovarian follicle, consists of three types of cells: an oocyte, granulosa cells, and theca cells that provide an appropriate developmental environment for oocytes (Gougeon, 1996). Growing ovarian follicles demonstrate a bidirectional communication between the oocyte and surrounding somatic cells that is essential for appropriate proliferation and differentiation (Nilsson and Skinner, 2001). These communications are regulated by several classical signaling pathways, including the Wnt, insulin, Notch, and hedgehog (HH) pathways (Li et al., 2021). Wnt signaling pathway activation by Wnt2 and Wnt4 plays a crucial role in normal ovarian follicle development by promoting granulosa cell proliferation (Boyer et al., 2010; Wang et al., 2010). The insulin signaling pathway regulates follicle development in the later stages of oocyte growth and promotes oocyte development by either upregulating the binding efficacy of LH to receptors or the number of receptors itself (Das and Arur, 2017). The Notch signaling pathway is known to regulate granulosa cell proliferation, since treatment with a Notch signaling inhibitor resulted in a significant decreased in their number (Jing et al., 2017). Lastly, the HH signaling pathway has been postulated to be a target signaling pathway in ovarian follicle development, since expression of HH ligands at the primary follicle stage is well established (Wijgerde et al., 2005).

A comprehension of the signaling pathways involved in ovarian development will help design better strategies for the generation of higher quality IVM oocytes. This article discusses the sonic hedgehog (SHH) signaling pathway, which has been reported to be involved in folliculogenesis and oocyte maturation. We have further placed a special emphasis on the application of SHH-induced IVM systems owing to the widespread use of IVM oocytes both in research and in the production of genetically engineered animals via assisted reproductive technologies.

**Hedgehog signaling pathway in the ovaries**

HH signaling was first discovered in *Drosophila* (Nüsslein-Volhard and Wieschaus, 1980) and functions by controlling gonadal and basic embryonic developmental processes via regulating cell proliferation, differentiation, and cell fate determination through morphogen gradients (Ingham and McMahon, 2001). Till date, mammals are known to possess three HH ligands, namely Indian (IHH), Sonic (SHH), and Desert (DHH) (Hooper and Scott, 2005), all of which are capable of binding to patched (PTCH), a transmembrane receptor. In the absence of HH ligands, PTCH blocks the activity of smoothened (SMO), a transmembrane signal transducer protein. Binding of HH ligands to PTCH relieves SMO inhibition, and further downstream signal transduction occurs through the activation of the transcription factor, glioma-associated oncogene homolog (GLI) (Hooper and Scott, 2005). The consequent transcription of target genes including *PTCH1* and *GLI1* in response to HH signaling regulates cell proliferation, migration, and differentiation during development (McMahon et al., 2003).

All components of the HH signaling pathway including the ligands (*IHH, SHH, and DHH*), the receptors (*PTCH1* and...
and PTCH2), the signal transducer (SMO), and downstream transcription factor (GLI1) are expressed in the mammalian ovary (Russell et al., 2007). In addition, treatment of granulosa cells with SHH protein enhances granulosa cell growth and proliferation with concomitantly elevated expression of GLI1, a transcription target of HH signaling, thereby indicating that granulosa cells may be potential targets of HH signaling (Russell et al., 2007). Recent studies have identified components of the HH signaling pathway including PTCH, SMO, and GLI1 in the granulosa layers of the ovary in various species, such as mice (Wijgerde et al., 2005), cattle (Spicer et al., 2009), and pigs (Nguyen et al., 2009). Since communication between oocytes and surrounding somatic cells such as granulosa cells and theca cells is essential for follicle development (Wijgerde et al., 2005), it may be hypothesized that HH signaling could be involved in folliculogenesis and oocyte maturation.

**Sonic hedgehog signaling pathway and in vitro oocyte maturation**

The SHH signaling pathway is a complex signal transduction mechanism that controls precisely regulated developmental processes (Choudhry et al., 2014). Signal transduction via this pathway is initiated by the binding of SHH protein in an autocrine and/or paracrine fashion (Handrigan and Richman, 2010) and ultimately coordinates cell proliferation and differentiation in various cell types (Enomoto-Iwamoto et al., 2000; Osawa et al., 2006; Saldaña et al., 2016). However, the evidence that the SHH signaling pathway controls the development of ovarian follicles is of particular interest (Russell et al., 2007), since folliculogenesis and oocyte maturation proceed together until ovulation (Kidder and Vanderhyden, 2010). Several studies have demonstrated an association between SHH signaling pathway and oocyte maturation in vitro (Table 1).

Treatment with recombinant SHH protein during IVM of pig oocytes was shown to promote nuclear maturation as well as pre-implantation embryonic development, which was reversed by the concomitant addition of cyclopamine, an SHH signaling inhibitor (Nguyen et al., 2009). Additionally, similar results on oocyte nuclear maturation were reported by Wang et al. by employing recombinant SHH protein and cyclopamine in caprine IVM medium (Wang et al., 2017). They further demonstrated that goat oocytes matured in the SHH protein supplemented medium demonstrated superior embryonic development both in vitro and in vivo (Wang et al., 2017). The results of these studies therefore directly imply that the SHH signaling pathway plays a key role in oocyte maturation.

The SHH signaling pathway can also be exploited as an indicator of oocyte quality. Previous reports have demonstrated that high-quality oocytes as assessed by brilliant cresyl blue (BCB) staining had greater potential to expand their surrounding cumulus cells along with reduced apoptosis and active SHH signaling (Lee et al., 2020). High-quality cumulus-oocyte complexes (COCs) in turn showed enhanced cumulus expansion, oocyte nuclear maturation, and preimplantation embryonic development. In addi-

| Supplement                  | Species | Effects                                                                 | Preimplantation embryonic development                                                                 | References                  |
|-----------------------------|---------|------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|-----------------------------|
| Recombinant SHH protein     | Pig     | Increased oocyte nuclear maturation, cyclin B1 content, ERK1/2 phosphorylation, and intracellular calcium release | Enhanced cleavage, blastocyst formation rates, and total cell number after PA                          | (Nguyen et al., 2009)       |
| Recombinant SHH protein     | Goat    | Increased oocyte nuclear maturation and ERK1/2 phosphorylation         | Enhanced blastocyst formation rate and in vivo development after IVF                                  | (Wang et al., 2017)         |
| Resveratrol                 | Pig     | Increased cumulus cell expansion, oocyte nuclear maturation, and the expression of SHH–related proteins (PTCH1, SMO, and GLI1) in both the oocyte and its surrounding cumulus cells | Enhanced cleavage, blastocyst formation rates, and total cell number after PA                         | (Lee et al., 2018)          |
| Melatonin                   | Pig     | Increased cumulus cell expansion and the expression of SHH–related proteins (PTCH1, SMO, and GLI1) in both the oocyte and its surrounding cumulus cells | Enhanced blastocyst formation rate and total cell number after PA                                       | (Lee et al., 2017)          |

ERK1/2, extracellular signal–regulated protein kinase 1/2; PTCH1, patched 1; SMO, smoothened; GLI1, glioma–associated oncogene homolog 1; PA, parthenogenetic activation; IVF, in vitro fertilization.
tion, they exhibited elevated expression of SHH signaling proteins including SHH, PTCH1, and GLI1 (Lee et al., 2020). The above findings collectively suggest that active signaling via the SHH pathway may be indispensable for the generation and/or maintenance of appropriate oocyte maturation environment.

Well-known antioxidants, such as resveratrol and melatonin, are known to activate the SHH signaling pathway in COCs and consequently improve oocyte maturation and subsequent embryonic development (Table 1). Lee et al. reported that the effect of resveratrol on cumulus cell expansion, oocyte nuclear maturation, and subsequent embryonic development in pigs is mediated via the SHH signaling pathway (Lee et al., 2018). Furthermore, improvement of cumulus cell expansion and subsequent embryonic development by melatonin has also been reported to occur through activation of SHH signaling (Lee et al., 2017). Given the relationship between SHH signaling pathway and oocyte maturation, it may be a suitable target for improving current IVM culture systems.

**CONCLUSION**

Recent studies have demonstrated the involvement of the SHH signaling pathway, which exists in ovarian follicles, in oocyte maturation (Fig. 1). In addition, it was also demonstrated that high quality oocytes have a greater potential to expand their surrounding somatic cells with active SHH signaling. Additionally, antioxidants including resveratrol and melatonin, improve oocyte maturation through SHH signaling activation. Therefore, activation of the SHH signaling pathway may aid in the establishment of an optimized IVM culture system by closely mimicking the in vivo environment of ovarian follicles.

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