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Review Article

Current approaches for assisted oocyte maturation in camels

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ABSTRACT Camel (camelus dromedarius) is a unique large mammalian species that can survive harsh environmental conditions and produce milk, meat, and wool. Camel reproduction is inferior when compared to other farm animal species such as cattle and sheep. Several trials have been reported to increase camel reproduction and production through assisted reproductive techniques (ARTs) such as in vitro fertilization and cloning. For these reasons, obtaining enough mature oocytes is a cornerstone for ARTs. This demand would be improved by the oocyte in vitro maturation (IVM) systems. In this review, the current approaches and views from different laboratories using ARTs and the IVM to produce embryos in vitro in camel species. For the last two decades, conventional IVM system was the common approach, however, recently the bi-phasic IVM system has been introduced and showed promising improvement in IVM of camel oocytes. Detailed studies are needed to understand camel meiosis and IVM to efficiently increase the production of this species.

Keywords: blastocyst, camel, cumulus cells, *in vitro* maturation, oocytes

INTRODUCTION

According to Food and Agriculture Organization (FAO), worldwide demand for meat will increase by 40% by the year 2050. To this end, several strategies, and approaches to increase the livestock population are being promoted. Reproductive success in livestock ensures the continuation, survival, and conservation of species, and by extension of food security through the availability of more food of animal origin. To maximize productivity and to address several reproductive challenges, assisted reproductive technologies (ARTs) have come to a rescue (Comizzoli et al., 2018; Herrick, 2019; Skidmore, 2019).

The dromedary camel (*Camelus dromedarius*), with its natural ability to produce quality meat, milk and fiber under very hot and most hostile climatic condition (Abri and Faye, 2019), is reproductively weak (Skidmore, 2003; Skidmore, 2019) and has received the least attention among livestock (Russo et al., 2014; Singh et al., 2019). Few important updates on ARTs in camel were recently reviewed by (Singh et al., 2019; Skidmore, 2019), and as stated, only few research centers are working on camel reproduction, and majorly the male camel. Even at that, camel oocyte maturation research is still fallow and the success rate of ARTs is still very slow and low (Abdelkhalek et al., 2017; Fathi et al., 2018; Moawad et al., 2020; Saadeldin et al., 2019; Yaqoob et al., 2017).

One major challenge that has not been researched in camel oocyte maturation is the spontaneous meiotic resumption. When immature oocytes leave the natural

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follicular environment and are cultured *in vitro*, they spontaneously resume meiosis because of absence of inhibitory signals (Edwards, 1965; Albuz et al., 2010). This sudden meiotic resumption causes loss of cumulus-oocyte communication (Wert and Larsen, 1989) which is crucial for oocyte health, metabolism, and acquisition of developmental competence (Barrett and Albertini, 2010; Tukur et al., 2020a). Eventually, oocytes will have a premature maturation and sadly, these prematurely developed oocytes, even though they appear morphologically normal, are not competent. This issue has been the subject of numerous earlier and recent studies (Lonergan et al., 2000; Gilchrist, 2011; Caballero et al., 2020).

Apart from the inherent fertility problem in the female camel, scarcity of camel ovary (and consequently, oocyte) partly contributes to slow development in camel IVM research. Since the slaughter of fertile female camel is restricted, this made some researchers to consider oocyte cryopreservation as option for *in vitro* embryo production (Fathi et al., 2018). However, a comprehensive study using freshly obtained camel oocytes for IVM research will help researchers to gain better understanding of camel oocyte *in vitro* maturation, develop standard protocol, and increase the rate of camel embryo production. Considering the gradual improvement and breakthrough in the IVM of other livestock species and human (Caballero et al., 2020; Vuong et al., 2020), would be a paradigm to improve camel IVM after previous work by various researchers (Mesbah et al., 2016; Yaqoob et al., 2017; Saadeldin et al., 2019).

Advances in *in vitro* oocyte maturation

In vitro maturation of oocyte was firstly demonstrated in 1935 by (Pincus and Enzmann, 1935) in an experiment involved rabbit oocytes. Interestingly, their first observation was the spontaneous meiotic resumption of oocytes after leaving the follicle. Their work clearly showed the possibility of obtaining successful maturation and fertilization of oocytes *in vitro*. All later IVM works were built upon their discovery. The report of Chang in 1955 further supported the earlier findings of Pincus (Chang, 1955). Significantly, the observation that oocyte maturation to the MII stage can be achieved without hormonal stimulation led to deeper investigation of IVM as an alternative and safer approach to IVF.

Inspired by Pincus's work, Edwards in 1956 further demonstrated IVM in different mammalian species including mice, pigs, cows, sheep, rhesus monkey and human (Edwards, 1965). Another significant advances recorded was the production of live mouse (Chang, 1955), and live human from the fertilization of *in vitro* grown oocytes (Cha et al., 1991). In domestic animals (goat, sheep, cattle, swine, and horse) production of embryos from *in vitro* matured oocytes has been a routine practice, but success rates are low (Kruip et al., 1991; Lonergan and Fair, 2016).

Another attempt to optimize oocyte development *in vitro* was the introduction of a two-step culture protocol by Eppig and O'Brien. This protocol involved *in vitro* culture of the ovary of newborn mice after isolation of the oocyte-granulosa complexes for a second culture. Unfortunately, only 0.5% of transferred embryos were survived (Eppig and O'Brien, 1996). This protocol was later improved (O'Brien et al., 2003).

It is hypothesized that this temporary delay of meiotic resumption could mimic the natural follicular environment and improve the developmental competence of *in vitro* matured oocytes (Caballero et al., 2020). Several approaches have been examined to delay oocyte meiotic resumption. These include blockage of cAMP signals, modulation of cAMP concentration (Lonergan et al., 2000; Albuz et al., 2010; Li et al., 2016; Soto-Heras et al., 2019a), inhibition of key molecules such as cyclin-dependent kinase 1 (CDK1) with roscovitine, and rho associated kinase with Y-27632 (Duan et al., 2014; Zhang et al., 2014; Zhang et al., 2017; Maziero et al., 2020; Zhu et al., 2020), use of adenosine (Caballero et al., 2020) and the inhibition of phosphodiesterase 3A by IBMX or C-type natriuretic peptide (Albuz et al., 2010; Sanchez et al., 2019; Soto-Heras et al., 2019a) and several other approaches.

Rho-associated protein kinases (ROCK) inhibitor, Y-27632, was used to improve blastocyst yield in human embryos (Huang et al., 2016). The Rho kinase is involved in many cellular functions and has been shown to be important for oocyte meiotic progression, and embryonic development (Arayatham et al., 2017). Short to long-term inhibition of ROCK activity was done and their consequent effects on camel oocyte maturation and gene expression were evaluated.

The sudden meiotic resumption that was earlier observed in IVM (Pincus and Enzmann, 1935; Edwards, 1965) causes loss of cumulus-oocyte communication (Wert and

Larsen, 1989) which is crucial for oocyte health, metabolism, and acquisition of developmental competence (Barrett and Albertini, 2010). It therefore became clear that *in vitro* matured oocytes suffer premature development and inability to undergo important physiological and morphological changes that are required to achieve competency (Assey et al., 1994). Hence, control of oocyte meiotic arrest and resumption could make IVM closer to the *in vivo* maturation. This prompted some researchers to develop IVM condition where oocyte meiotic arrest is maintained at the GV stage i.e. preventing spontaneous maturation (Sirard, 1990; Fulka et al., 1991; Lonergan et al., 1997; Mermillod et al., 2000). Although meiotic arrest was achieved, the inadequacy of this method was that the subsequent development of the oocytes after the period of inhibition was not reported (Lonergan et al., 1997).

The biphasic IVM approach involves temporary delay in meiosis resumption (holding the oocyte at GV stage *in vitro*) in a pre-maturation phase using meiotic inhibitors before submission to normal *in vitro* maturation (Lonergan et al., 2000; Sanchez et al., 2019). This is an improvement on the previous methods of sustaining meiotic arrest in IVM (Caballero et al., 2020).

As stated earlier, the dromedary camel has low reproductive efficiency and efforts to improve this is slow and low compared to other species (Skidmore, 2003; Singh et al., 2019; Skidmore, 2019). Few research on IVM of dromedary camel oocytes have been reported and it is still at the early stage (Yaqoob et al., 2017; Fathi et al., 2018; Saadeldin et al., 2019). One of the areas that requires in-depth research in camel oocyte maturation is the spontaneous meiotic resumption which is a common challenge in IVM of all species. Since this area is barren in camel oocyte research, it is therefore the focus of this thesis. Table 1 summarizes the approaches used for camel IVM using either single phase or bi-phasic IVM system.

CONCLUSION AND FUTURE PERSPECTIVES

Since the recognition of *in vitro* maturation as a simple, promising, non-invasive, and cheaper procedure for embryo production in ARTs, extensive research has been conducted on different species. Among livestock, because both bovine and porcine have received most attention in

Table 1. The current approaches for camel cumulus-oocyte complex in vitro maturation (IVM)

IVM type	Main supplement	Effects	In vitro embryo development	References
Single phase IVM	10 % fetal calf serum	Optimizing IVM duration	NA	Kafi et al., 2005; Abdoon et al., 2011
	10 % estrous camel serum	Optimizing ovary storage duration	NA	Wani and Nowshari, 2005
	All-trans retinoic acid	Increased TGFβ expression in cumulus cells and increased MII oocytes	No effect on blastocyst % after PA	Saadeldin et al., 2019
	10 % follicular fluid	Improved cumulus expansion and MII oocytes	No effect on blastocyst % after PA	Yaqoob et al., 2017
	15 % fetal calf serum	Optimizing IVM duration	NA	Russo et al., 2014
	20 ng/mL EGF	Optimizing IVM Improved MII oocytes	NA	Wani and Wernery, 2010
	PVA	Optimizing IVM Improved MII oocytes	Improved blastocysts after PA	Moulavi and Hosseini, 2019
	25 μM melatonin	Improved MII oocytes	Improved blastocysts after IVF	Fathi et al., 2021
	10 mM caffeine	Improved MII oocytes	Improved blastocysts after IVF	Fathi et al., 2014
	0.5 mg/mL L-carnitine	Improved MII oocytes	Improved blastocysts after IVF	Fathi and El-Shahat, 2017
	50 μg/mL of vitamin C	Improved MII oocytes	NA	Al-Malikey and Al-Delemi, 2021
Biphasic IVM	50 μM Roscovitine for 24 h	GVBD inhibition	Increased blastocyst formation after PA	Wani and Hong, 2020
	10 μM ROCK inhibitor (Y-27632) for 4 h	Improved cumulus expansion, reduced apoptosis, and increased MII oocytes	NA	Tukur et al., 2020b

GVBD, Germinal vesicle breakdown; IVF, In vitro fertilization; MII, meiotic II, 1st polar body extrusion; NA, Not applicable; PA, parthenogenetic activation; ROCK, Rho-associated protein kinases.

this area (Bahrami et al., 2019) and in the recent years, notable improvement and advances in IVM technique have been demonstrated. The different biphasic IVM methods reported in recent studies have recorded amazing outcomes in human (Sanchez et al., 2019; Vuong et al., 2020) and livestock species (Soto-Heras et al., 2019b; Caballero et al., 2020), and recently in camels (Tukur et al., 2020b; Wani and Hong, 2020). Further investigations about camel meiosis is required to establish an efficient *in vitro* maturation system in this unique species.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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Conceptualization: IMS and JC
Investigation: IMS and JC
Methodology: IMS and JC
Writing – original draft: IMS and JC
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