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Review

Current status and future direction of cryopreservation of camelid embryos

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ABSTRACT

Over the past 3 decades, and similar to the horse industry, fresh embryo transfer has been widely practiced on large commercial scales in different camelid species, especially the dromedary camel and alpaca. However, the inability to cryopreserve embryos significantly reduces its broader application, and as such limits the capacity to utilize elite genetic resources internationally. In addition, cryopreservation of the semen of camelids is also difficult, suggesting an extreme sensitivity of the germplasm to cooling and freezing. As a result, genetic resources of camelids must continue to be maintained as living collections of animals. Due to concerns over disease outbreaks such as that of the highly pathogenic Middle East Respiratory Syndrome in the Middle East and Asia, there is an urgent need to establish an effective gene banking system for camelid species, especially the camel. The current review compares and summarizes recent progress in the field of camelid embryo cryopreservation, identifying four possible reasons for the slow development of an effective protocol and describing eight future directions to improve the current protocols. At the same time, the results of a recent dromedary camel embryo transfer study which produced a high morphologic integrity and survival rate of Open Pulled Straw-vitrified embryos are also discussed.

1. Introduction

The camelid family includes dromedary and Bactrian camels, llamas, alpacas, vicunas, and guanacos. The first two are Old World camelids, whereas the last four are known as New World camelids or South American camelids. Camelids are strictly herbivorous animals and have unique reproductive characteristics [1–3]. They are seasonal breeders, the females only ovulate postmating (referred to as induced ovulators), and males produce viscous semen. Interestingly, all of these species have the same number of chromosomes (37 pairs), and interspecies crossbreeding can generate hybrids [4–6].

The reproductive efficiency of camelids is low partly due to the late onset of puberty, the short breeding season, early embryonic loss, and a long gestation period of 13 months. Accordingly, assisted reproduction technologies, such as fresh embryo transfer (ET), have been widely practiced in dromedary camel breeding programs in Middle Eastern countries [7], and in alpaca and llama programs mainly in Australia and South America [8,9]. However, frozen embryos are not used in those commercial operations due to an unacceptably low pregnancy rate [1,10]. The ability to successfully cryopreserve embryos could overcome the spatial and temporal barriers between recipient and donor, and subsequently offer considerable logistical and economic advantage. This is especially important for camelids because recipients need to have certain sized follicles

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(growing follicles) to be ovulated with the treatment of hormones, and this requires a large pool of females from which to select appropriate recipients [11]. In addition, embryo cryopreservation provides an effective means of preserving endangered camelids, such as the vicuna and guanaco. Furthermore, a successful cryopreservation technology would promote the application of other embryo biotechnologies, such as cloning and transgenics, on large commercial scales [12]. Thus, there is a need to compare and summarize recent progress in the field of embryo cryopreservation for camelids to provide researchers with new insight into designing experiments that will lead to more effective cryopreservation protocols for use in ET programs to facilitate a wider application.

To review and cover all results published in the field, literature searches were conducted for each species and each technique using the PubMed database. Key word combinations used were as follows:

I. Camelids, embryo, cryopreservation or freezing,
II. Dromedary, embryo, cryopreservation or freezing,
III. Bactrian, embryo, cryopreservation or freezing,
IV. Alpaca, embryo, cryopreservation or freezing,
V. Ilama, embryo, cryopreservation or freezing.

A total of 22 articles were retrieved and reviewed, and with the current authors' extensive experience in cryopreservation of embryos and stem cells in the human [13], bovine and camel [14–16], several approaches on how to improve the efficacy of the existing protocols for freezing camelid embryos are presented.

1.1. History of cryopreservation of camelid embryos

Two approaches of cryopreservation, slow freezing and vitrification, are most commonly used to maintain functional capacity of animal germplasm during a cooling and warming process. Although cryopreservation of germplasm has been successfully applied in human medicine and to some livestock breeding programs [15,17], the cryopreservation of camelid embryos is in its infancy (Table 1), with the focus on modification of established protocols commonly used for other species [18,27].

Attempts to freeze camelid embryos started in the late 1990s, with the application of ET to dromedary camels [3,18]. As shown in Table 2, a number of pregnancies from frozen/thawed ETs have been reported in dromedary camels [18] and llamas [8], with two live births from vitrified embryos [10,16] and one from a slow-frozen embryo in dromedary camels [18]. It has been nearly 3 decades since the initial studies, but embryo cryopreservation has still not yet been successfully incorporated into an ET program, a reflection of the difficulties associated with developing an effective procedure for camelids.

Possible reasons for the slow development of an effective cryopreservation protocol for camelids include the following:

I. The lack of zona pellucida in hatched embryos: The permeability of cryoprotective agents (CPAs) during the cooling/warming processes might be influenced by the lack of zona pellucida in hatched embryos [12]. In the current ET practice with dromedary camels, example, hatched embryos are preferably collected on Days 7, 8, or 9 after ovulation with the intention of enhancing the recovery rate. Therefore, the protocols developed for nonhatched embryos in other species are unsuitable for freezing camelid hatched embryos.
II. A much larger variation in embryo size: Embryo size not only differs between donors on Days 6, 7, and 8 but it can also vary substantially between embryos harvested from one animal. Thus, there is a challenge to develop protocols that fit different-sized embryos [27].
III. A great amount of lipids in embryos: Similar to porcine embryos, camelid embryos contain a high concentration of lipids—this has been shown to have an adverse effect on conventional freezing methods [28,29].
IV. The lack of a convenient and reliable evaluation system for embryo quality: The morphologic appearance of cryopreserved embryos does not always correlate to their developmental potential, and so, it is insufficient to assess the outcome of the cryopreservation and to predict ET success [27].

1.2. Slow freezing

The principle of cryopreservation is to use permeating CPAs (e.g., glycerol, propanediol [PROH], DMSO, and ethylene glycol [EG]) and nonpermeating CPAs (e.g., sucrose, glucose, and trehalose) to replace intracellular water from embryos and prevent the formation of ice crystals

Table 1
History of cryopreservation of camelid embryos and its comparison with other domestic species.

Species	Method	Achievement	Years	Reference of first report
Dromedary camel	Slow freezing	Pregnancy/live birth	2002	Skidmore et al. [18]
	Vitrification	Pregnancy/live birth	2005	Skidmore et al. [10] and Nowshari et al. [16]
Lama	Slow freezing			
	Vitrification	Pregnancy	2002	Aller et al. [8]
Bovine	Slow freezing	Live birth	1973	Wilmut and Rowson [19]
	Vitrification	Live birth	1986	Massip et al. [20]
Ovine	Slow freezing	Live birth	1976	Willadsen et al. [21]
	Vitrification	Live birth	1994	Széll et al. [22]
Swine	Slow freezing	Live birth	1989	Hayashi et al. [23]
	Vitrification	Live birth	2000	Dobrinsky et al. [24]
Horse	Slow freezing	Live birth	1982	Yamamoto et al. [25]
	Vitrification	Pregnancy	2005	Eldridge-Panuska et al. [26]