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Recent advances toward the practical application of embryo transfer in pigs

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ABSTRACT

Porcine embryo transfer (ET) technology has been in demand for decades because of its potential to provide considerable improvements in pig production with important sanitary, economic, and animal welfare benefits. Despite these advantages, the commercial use of ET is practically nonexistent. However, the two main obstacles hindering the commercial use of ET in pigs in the past several decades (i.e., surgical transfer and embryo preservation) have recently been overcome. A technique for nonsurgical deep-uterine (NsDU) ET of nonsedated gilts and sows, which was seemingly an impossible challenge just a few years ago, is a reality today. The improvements in embryo preservation that have been achieved in recent years and the excellent reproductive performance of the recipients after the NsDU-ET technique coupled with short-term and long-term–stored embryos represent essential progress for the international trade of porcine embryos and the practical use of ET by the pig industry. This review focuses, with an emphasis on our own findings, on the recent advances in embryo preservation and NsDU-ET technologies, which are starting to show potential for application under field conditions.

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1. Introduction

There is great interest in the use of embryo transfer (ET) in pig production because of its broad applications, including the movement and introduction of new genetic material (i.e., embryos) into a herd with reduced transportation cost, absence of an effect on animal welfare during transport, and minimal risk for disease transmission. Despite these advantages, the commercial use of ET in pigs is currently practically nonexistent. The main reasons for the limited use of ET in this species have been the requirement for surgical procedures to transfer the embryos to recipients and the difficulties in cryopreserving the embryos. However, in the past decade, new methodologies have been developed that enable successful nonsurgical ET and efficient embryo preservation.

Although the first pregnancy in pigs through nonsurgical ET was reported almost 50 years ago [1], nonsurgical ET was considered an impossible technique for many years because of the complex anatomy of the swine genital tract. However, in the 1990s, several nonsurgical techniques for depositing embryos directly into the uterine body were developed, but none of them were sufficiently successful (reviewed in [2,3]). To overcome some of the physiological and practical limitations of nonsurgical uterine body ET, we developed a new procedure for the nonsurgical deep-uterine (NsDU) ET of nonsedated gilts and sows. During the first attempt at NsDU-ET using fresh embryos, an acceptable reproductive performance of the recipients was achieved [4]. With improvements in the procedure, the results were greatly enhanced [5,6], even when fresh embryos cultured for 24 hours were used [7].

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Although it is possible to routinely cryopreserve embryos from several mammalian species, the cryopreservation of pig embryos has largely been limited because of their high sensitivity to chilling injury [8,9]. At present, vitrification is considered the only suitable method for the long-term storage of porcine embryos. The substantial progress achieved over the past 15 years in vitrification protocols has resulted in high postwarming *in vitro* survival of morulae and blastocysts even without embryo pretreatments, such as delipidation, cytoskeletal stabilization, or centrifugation (hereafter referred to as "untreated embryos"). Moreover, promising farrowing rates and litter sizes have been reported after the surgical and NsDU transfer of vitrified embryos (reviewed in [3]). However, the number of studies is limited, and these studies have involved low numbers of ETs, which may have been due to the effort and high costs required to obtain large numbers of embryos of this species.

Although the use of vitrified embryos coupled with NsDU-ET could be essential for the widespread use of porcine ET, other alternatives for embryo preservation (e.g., short-term [24–48 hours] storage of fresh embryos) should also be considered. Here, we provide a brief summary of the current achievements in porcine embryo preservation and describe some basic aspects of NsDU-ET technology. Finally, we discuss several factors that affect the success of NsDU-ET for its practical application. This review will only refer to the *in vivo*–derived embryos because from a practical and commercial point of view, they are the only embryos with potential short-term application in pig production.

2. Embryo preservation

After collection, the embryos must be stored until they are transferred to the recipients. There are several methods of preserving the embryos. *In vitro* culture can be used as a method for medium- to short-term embryo storage, whereas vitrification is the only efficient method available for the long-term preservation of pig embryos.

2.1. Medium- to short-term storage of embryos

A potential method to maintain the developmental capacity of the embryos for brief periods is IVC, which can be used as a method for medium-term (72–120 hours) or short-term (24–48 hours) embryo storage.

For medium-term storage, the embryos must be collected at a very early developmental stage, which prevents development beyond the unhatched blastocyst stage at the end of the culture. This issue is essential because embryos must be protected by an intact ZP for sanitary reasons [10]. High blastocyst formation rates from one-, two-, and four-cell embryos cultured *in vitro* for 72 to 120 hours have been reported [11,12]. Although these blastocysts had lower cell numbers compared with their *in vivo* counterparts, no difference between the two types of blastocysts was noted in the *in vivo* developmental ability after surgical ET [13,14]. Because of the limited number of studies, more research is needed to evaluate the effectiveness of different culture media and temperatures on the *in vitro* and *in vivo* development of medium-term–stored embryos.

To minimize the detrimental effect of the culture conditions on embryo quality, a shorter culture period, such as 24 hours, can be used. A period of 24 hours between the collection and transfer should be sufficient for the regional, national, and even international transportation of the embryos to their recipients. Despite its importance, research on short-term porcine embryo culture has been limited. Using this storage method, acceptable farrowing rates (50%–60%) and litter sizes (5–8 piglets born) have been obtained after surgical transfers of embryos cultured for 24 to 30 hours [15,16], which indicates that short-term–cultured embryos are able to develop to term. In addition, several types of serum-containing or BSA-containing media and several temperatures have been shown to be effective for short-term embryo culture [17], although the *in vivo* developmental capacity after the transfer of the cultured embryos was not evaluated. In a recent study, we achieved high reproductive performance in the recipients after NsDU-ET using fresh cultured morulae kept at 37 °C for 24 hours in a chemically defined medium [7]. The results of this study indicated that Tyrode's lactate (TL)-HEPES-polyvinyl alcohol (TL-PVA) provided a chemically defined medium capable of maintaining a high *in vitro* viability of porcine morulae cultured at 37 °C for 24 hours. More than 95% of the embryos cultured under these conditions progressed to the unhatched blastocyst stage during culture (Fig. 1), and, unlike the controls, none of them hatched at the end of the culture, which is, as noted previously, essential for sanitary reasons. Although culture caused certain embryo developmental delays, the resulting blastocysts retained their potential to develop to term in the same manner as uncultured blastocysts (Fig. 2). Interestingly, in that study, for the first time, the embryos could be collected, handled, cultured, and transferred in the defined TL-PVA medium. This fact is essential because embryo culture media usually contain serum or serum components, which carry a risk of disease transmission [18], an important limitation for embryo transport.

Currently, we are evaluating the potential to increase the storage period of fresh morulae and blastocysts up to 48 hours by using various culture media and temperatures. Although the *in vivo* development of these embryos cultured for 48 hours has not yet been fully investigated, the preliminary *in vitro* results are promising. Moreover, for morulae and early blastocysts, we are evaluating the potential to prolong the storage period to 72 hours after embryo collection because this would enable worldwide embryo transport and transfer. The use of short-term embryo storage in combination with NsDU-ET technology opens new possibilities for the sanitary and safe national and international trade of fresh porcine embryos as well as the practical application of ET in pigs under field conditions.

2.2. Long-term storage of embryos

Although a short-term culture period may permit the international transport of embryos, cryopreservation of embryos is preferable. Embryo cryopreservation allows for

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