REPRODUCTION
ANNIVERSARY REVIEW

Intracytoplasmic sperm injection in domestic and wild mammals

Daniel F Salamone, Natalia G Canel and María Belén Rodríguez

Laboratorio de Biotecnologia Animal, Facultad de Agronomia, Universidad de Buenos Aires-CONICET, Buenos Aires, Argentina,

Correspondence should be addressed to D F Salamone; Email: salamone@agro.uba.ar

This paper is part of an Anniversary Issue celebrating 25 Years of ICSI. The Guest Editor for this section was Professor Gianpiero Palermo.

Abstract

Intracytoplasmic sperm injection (ICSI) has become a useful technique for clinical applications in the horse-breeding industry. However, both ICSI blastocyst and offspring production continues to be limited for most farm and wild species. This article reviews technical differences of ICSI performance among species, possible biological and methodological reasons for the variable efficiency and potential strategies to improve the outcomes. One of the major applications of ICSI in animal production is the reproduction of high-value specimens. Unfortunately, some domestic species like the bovine show low rates of pronuclei formation after sperm injection, which led to the development of various artificial activation protocols and sperm pre-treatments that are discussed in this article. The impact of ICSI technique on equine breeding programs is considered in detail, since in contrast to other species, its use for elite horse reproduction has increased in recent years. ICSI has also been used to produce genetically modified animals; however, despite numerous attempts in several domestic species, only transgenic pigs have been consistently produced. Finally, the ICSI is a promising tool for genetic rescue of endangered and wild species. In conclusion, while ICSI has become a consistent ART for some species, it needs further development for others. The low results obtained for some domestic species, the high training needed and the equipment required have limited this technique to the production of elite specimens or for research purposes.

Reproduction (2017) **154** F111–F124

General overview

ICSI is a micromanipulation technique that involves the injection of a single spermatozoon into the cytoplasm of a mature oocyte. The first report of pronuclei formation after ICSI in mammals was achieved in hamster gametes (Uehara & Yanagimachi 1976). In 1992, the first baby generated by sperm injection was born (Palermo *et al.* 1992), and it did not take long for ICSI to become an important technique for human-assisted reproduction worldwide (reviewed by Devroey & Van Steirteghem 2004). Following that attainment, the use of this technique extended to other species, including the cow (Goto *et al.* 1990), rabbit (Hosoi & Iritani 1993), mouse (Kimura & Yanagimachi 1995), sheep (Catt *et al.* 1996), horse (Cochran *et al.* 1998), domestic cat and wild felids (Pope *et al.* 1998), pig (Kolbe & Holtz 2000) and goat (Wang *et al.* 2003).

However, despite the efforts of several working groups around the world, the success of this technique has been limited in farm animals. The most extreme case is the cow, whose fertilization rates after ICSI are critically low (Chung *et al.* 2000, Devito *et al.* 2010, Arias *et al.* 2014). In sheep, although fertilization rates after ICSI can be improved by artificial activation treatments (Shirazi *et al.* 2011), development to blastocyst continues to be low. In regard to post-implantation development, the number of newborns produced by ICSI remains extremely low for these species (reviewed by Garcia-Rosello *et al.* 2009, Lopez-Saucedo *et al.* 2012).

In contrast to the situation for most domestic species, ICSI in horses has developed to a commercial level (Hinrichs 2005). In this species, embryo production by IVF continues to be a challenge, since it has not been possible to obtain repeatable results (Mugnier *et al.* 2009, reviewed by Leemans *et al.* 2016). For this reason, the combination of ovum pick-up (OPU) and ICSI followed by non-surgical embryo transfer to recipient mares is the current routine protocol for *in vitro* embryo production in horses. In recent years, surprising efficiency of this protocol ended in its inclusion in commercial breeding programs (Galli *et al.* 2014).

With regard to pigs, ICSI became an alternative fertilization technique for research purposes, since IVF produces high rates of polyspermia (reviewed by Coy & Romar 2002). Moreover, after the generation of the first transgenic piglet by sperm injection, ICSI gained importance as a new tool for inducing genetic

ISSN 1470–1626 (paper) 1741–7899 (online)
https://doi.org/10.1530/REP-17-0357
Online version via www.reproduction-online.org

modifications in farm animals (Kurome *et al.* 2006, Garcia-Vazquez *et al.* 2010). However, the advent of CRISPR-Cas9 system for genetic engineering lead to the replacement of ICSI by regular or IVF zygotes for the generation of genetically modified animals (Hai *et al.* 2014, Whitworth *et al.* 2014, Proudfoot *et al.* 2015, Wang *et al.* 2015, Bevacqua *et al.* 2016).

In summary, the low efficiency of ICSI in domestic species, the high level of training needed, and the expensive equipment required has restricted this technology to the production of specimens of high commercial value or for research purposes. The great progress achieved by the development of this technique in some species, and the disappointing results observed in others emphasize the importance of re-examining the possible causes of such differences, encouraging the study of early fertilization events and considering new applications that have not been explored thoroughly yet.

The technique step by step

The ICSI procedure involves the use of complex equipment, including an inverted microscope coupled to a micromanipulation system. Basically, the micromanipulator converts macroscopic movements into microscopic ones, allowing the handling of gametes. It is equipped with two arms, one attached to a holding pipette and the other to an injection pipette that, in some cases, is connected to a piezo-driven system. The holding pipette attaches the oocyte, placing the first polar body (PB) in 6 or 12 clockwise position. The injection pipette, used for immobilizing and holding a single spermatozoon, will pass through the membrane of a metaphase II oocyte and deposit the sperm into the cytoplasm. Since the genetic material is expected to be next to the PB, it is kept far from the area of injection, in order to minimize the risk of chromosome or spindle damage.

Several authors have described in detail the methodology of ICSI technique (Yoshida & Perry 2007, Stein & Schultz 2012, Rader *et al.* 2016, Simopoulou *et al.* 2016). In the present section, we expose technical differences among species on the basis of our collective experience. These differences are evidenced during the ICSI procedure. After *in vitro* maturation (that will vary between 18 and 30 h depending on the species), cumulus cells are removed from COCs through enzymatic treatment followed by vortexing or gentle pipetting. For example, when manipulating horse or wild animal's oocytes, vortexing is usually avoided to minimize the risk of losing or damaging them, given their high value.

Regarding sperm sample preparation frozen-thawed semen can be used, even for species with variable sperm freezability among individuals like horses (Hoffmann *et al.* 2011) and pigs (Casas *et al.* 2009). Depending on the quality of the sperm sample and the species used, methods for selection of motile spermatozoa like swim up or density gradient separation can be included on ICSI protocols (Gomez *et al.* 1997, Keskintepe *et al.* 1997, Choi *et al.* 2002, Nakai *et al.* 2016*a*,*b*, Rader *et al.* 2016). It is interesting to note the possibility of optimizing the use of frozen semen straws when performing ICSI. Since only one spermatozoon per injected oocyte is needed, each straw can be sectioned into multiple 'ICSI-cuts'. Up to ten 'ICSI-cuts' can be obtained from a single straw that can be thawed separately for its use in differed ICSI procedures (Rader *et al.* 2016). Additionally, some authors maximize the use of valuable straws not only by using this strategy, but also by diluting and refreezing sperm doses, for example, when a frozen semen store is limited or when expensive sex-sorted sperm straws are employed (Hamano *et al.* 1999, Rader *et al.* 2016, Canel *et al.* 2017).

After semen thawing and selection, a critical step is sperm immobilization. For this, the spermatozoa must be placed in a polyvinylpyrrolidone (PVP) droplet, a solution of high viscosity that reduces sperm motility (Hyakutake *et al.* 2015). The slow movement of the sperm in PVP allows placing the injection pipette over the sperm tail and rolling it against the bottom of the ICSI dish to immobilize the sperm and easily take it into the ICSI pipette. The resulting damage of the sperm tail membrane not only makes sperm manipulation simpler (Kato & Nagao 2009), but also is thought to facilitate sperm head decondensation and oocyte activation, which are essential steps for early embryo development (Morozumi *et al.* 2006). The resistance of the sperm tail to be broken varies among species, being much higher for the bull, followed by the sheep, the pig and finally horses and domestic cats, whose sperm tails are easily broken. Regardless of motility, even when dead spermatozoa are used, sperm tail breakage is a step that should not be bypassed, since it was shown to improve sperm nucleus decondensation (Dozortsev *et al.* 1995).

With respect to the pipettes used, commercial or handmade models can be employed. Since sperm size varies among species, it must be taken into account that injection pipettes of different inner diameters should be used in each case. For example, we recommend the use of 9 µm inner diameter pipettes for bull, ram (Fig. 1B) and pig sperm injection. On the other hand, commercial pipettes employed for human reproduction (7 µm) can be used for horse (Fig. 1A) and cat sperm injection (Fig. 1C). Furthermore, the shape of the pipette will depend on the system used. While sharp pipettes with bevel and spike are employed for the conventional method or laser-assisted system (Smits *et al.* 2012*a*), but a blunt pipettes are used when a piezo-driven system is employed. The piezoelectric actuator couples to the micromanipulation system and attaches the injection pipette, driving its tip forward in a precise and fast movement. In this fashion, disruption of the sperm tail oolemma are performed mechanically rather than manually, rendering the procedure easier, and more

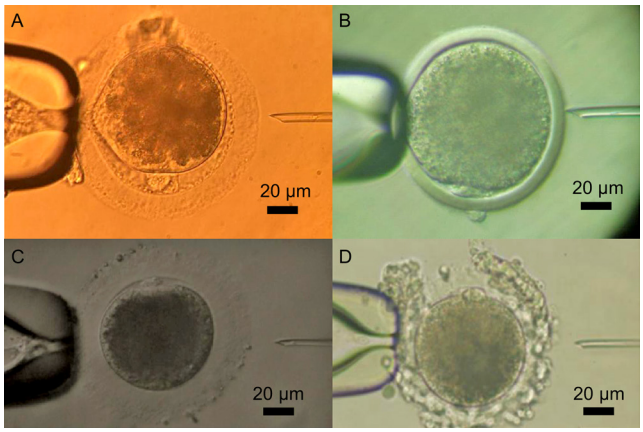

Figure 1 Pipette set up and oocyte of different species. (A) Equine oocyte, (B) ovine oocyte, (C) domestic cat oocyte and (D) leopard oocyte. PB, polar body.

successful according to some authors (Huang *et al.* 1996, Choi *et al.* 2002, Lazzari *et al.* 2002, Wei & Fukui 2002, Yoshida & Perry 2007). Penetration of the oolemma is another critical step. If a piezoelectric actuator is used, the procedure is greatly simplified (Horiuchi *et al.* 2002). Otherwise, when a piezoelectric system is not available, the difficulty of the procedure will vary depending on the elastic properties of the oocyte membrane, which differs between species. In our experience, cow oolemma offers the greatest resistance to injection. In the case of sheep, the operator must be very careful, since oocytes are more sensitive to handling, as reflected in higher lysis rates after injection. When conventional ICSI is performed, the oolemma must be penetrated manually. To achieve this, aspiration must continue until a speed change of the ooplasm entry rate inside the injection pipette is observed. Only then the spermatozoon can be injected into the oocyte, along with the previously aspirated ooplasm. Failed injection of oocytes is very common among untrained operators, due to the skipping of this critical step. In addition, the high lipid content the ooplasm of many of domestic species hinders the visualization of the pipette and the entry of the spermatozoon. Lipids confer an opaque appearance to oocytes, which is more intense in pigs and domestic cats, followed by cows and finally sheep and goats, whose ooplasm is clearer. In the case of horses, lipids polarization is commonly observed, which might facilitate the visualization of the spermatozoon within the oocyte. Finally, oocyte activation is such an important step of ICSI protocols for domestic species that it will be discussed in a separate section.

Oocyte activation induced by regular fertilization and sperm injection

After sperm–egg fusion in regular fertilization, the spermatozoon triggers oocyte activation, giving rise to early embryo development. The complete activation of the oocyte implies the resumption of meiosis, the second PB (2PB) extrusion, the release of cortical granules and the formation of male and female pronuclei (PN) (reviewed by Alberio *et al.* 2001, Swann & Lai 2016). Oocyte activation occurs as the result of Ca2+ oscillations in the ooplasm (Stricker 1999), and it is widely accepted that the factor responsible for triggering these oscillations in mammals is a sperm-specific isoform of the phospholipase C, named PLCζ (Saunders *et al.* 2002, Yoon & Fissore 2007). The currently accepted model is that PLCζ enters the ooplasm after the fusion of both gametes and catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate, generating inositol 1,4,5-triphosphate (IP3) and diacylglycerol. IP3 binds to its receptor in the membrane of the endoplasmic reticulum and induces the release of Ca^{2+} to the cytosol. In this fashion, PLCζ induces successive ooplasmic Ca^{2+} peaks (reviewed by Malcuit *et al.* 2006*a*). In mammals, such Ca^{2+} oscillations must be strictly regulated to induce a correct oocyte activation and normal embryo development (Rogers *et al.* 2006). Further in the cascade, Ca^{2+} peaks cause a decrease in the levels of maturation-promoting factor (MPF) and mitogen-activating protein kinase (MAPK), whose concentrations are at their maximum prior to fertilization, inducing oocyte activation (reviewed by Ducibella *et al.* 2002, Jones 2005).

For species like the human, mouse, horse and domestic cat, the sole injection of the sperm into the oocyte is enough to trigger oocyte activation, sustaining development to blastocyst, and even to term (Palermo *et al.* 1992, Cochran *et al.* 1998, Gomez *et al.* 2000, Kimura & Yanagimachi 1995). In human and mouse oocytes subjected to sperm injection, Ca^{2+} oscillations similar to those of IVF embryos have been observed (Tesarik *et al.* 1994, Sato *et al.* 1999, Markoulaki *et al.* 2007). In horses and domestic cats, although there are no reports comparing oscillation patterns of ICSI vs IVF embryos, empirical data suggest that the sole sperm injection stimulus would be enough to induce embryo development, since most ICSI embryos are able to cleave, and some of them reach the blastocyst stage (Pope *et al.* 1998, Bedford *et al.* 2003, Tharasanit *et al.* 2012, Moro *et al.* 2014). Indeed, 43–74% of horse oocytes formed pronuclei after sole sperm injection (Dell'Aquila *et al.* 2001, Tremoleda *et al.* 2003, Lewis *et al.* 2016). For this reason, it is not necessary to employ additional protocols to induce oocyte activation and subsequent embryo development. Nevertheless, it is not the case for some large domestic species, whose development to blastocyst and even more to term is extremely low (reviewed by Horiuchi & Numabe 1999).

Developmental rates of cow ICSI embryos are much lower compared to those produced by IVF (Goto *et al.* 1990, Chung *et al.* 2000). More than 90% of oocytes are unable to perform Ca^{2+} oscillations after sperm injection

(Malcuit *et al.* 2006*a*), resulting in an incomplete inactivation of MPF (Fujinami *et al.* 2004). Therefore, most ICSI cow embryos show inconsistencies in sperm decondensation and pronuclei formation (Rho *et al.* 1998*a*, Chung *et al.* 2000, Malcuit *et al.* 2006*b*, Arias *et al.* 2015), making ICSI success in a complex challenge. It is under discussion if such inconsistencies are due to the inability of bull sperm to induce the complete oocyte activation or to the poor response of cow ooplasm to injection stimulus, which provoke an incorrect sperm head decondensation (Aguila *et al.* 2017). For this reason, several oocyte activation protocols and sperm pretreatments have been developed, in order to improve releasing and/or activation of PLCζ and the complete inactivation of MPF and MAPK (see below). Although, there are a few exceptions, there is a general consensus about the need to artificially activate ICSI embryos to generate blastocysts in the cow (Rho *et al.* 1998*a*, Chung *et al.* 2000, Fujinami *et al.* 2004, Oikawa *et al.* 2005, Bevacqua *et al.* 2010).

After ICSI in sheep, development to blastocyst is low compared to IVF (Gomez *et al.* 1998*a*,*b*). In contrast to bovine ICSI, it is not due to failed pronuclei formation, but to the arrest of most ICSI embryos at the 8- to 16-cells stage (Gomez *et al.* 1998*a*). In our hands, cleavage rates increase from 53 to 85% after chemical activation, but no differences were observed at later stages (Pereyra Bonnet *et al.* 2008).

For pig embryos, although the need of artificial activation after ICSI continues to be controversial (Kikuchi *et al.* 2002, Yong *et al.* 2006, Li *et al.* 2013), several authors improved blastocyst rates through the employment of activation treatments (Lee *et al.* 2003, Nakai *et al.* 2003, 2006, Probst & Rath 2003). Indeed, it was recently reported that 50% of pig oocytes do not show Ca^{2+} oscillations after ICSI (Nakai *et al.* 2016*a*). However, in this particular species, the main problem is the absence of *in vitro* maturation and culture systems specially designed for pig oocyte and embryo requirements, that led to poor-quality blastocysts and low rates of *in vitro* and *in vivo* development (Garcia-Rosello *et al.* 2009, Li *et al.* 2013, Nakai *et al.* 2016*b*). In this sense, domestic cat ICSI embryos are in a similar situation. Although most injected oocytes are activated by sperm injection stimulus, only a few are capable of reaching the blastocyst stage. In this species, the problem probably lies on inadequate maturation, since developmental competence of ICSI and IVF embryos was shown to be lower for *in vitro*-matured oocytes compared to their *in vivo* counterparts (Gomez *et al.* 2000).

Two strategies for improving pronuclei formation

As discussed earlier, cow oocytes are not effectively activated by sperm after ICSI. In contrast to other animal groups, the great economical interest of this species has led to the development of *in vitro* production systems highly adapted to cow embryo requirements. However, male pronucleus formation after ICSI continues to fail (Wei & Fukui 1999, Suttner *et al.* 2000, Sekhavati *et al.* 2012). For these reasons, in the present section, we will mainly refer to bovine outcomes for describing the approaches assessed in order to improve pronuclei formation. Two types of strategies have been implemented: one focuses on the oocyte, by the use of exogenous activation treatments to induce early embryo development, and the other focuses on damaging the sperm membrane through the use of various pre-treatments in order to emulate, as much as possible, regular fertilization events.

Regarding activation protocols, they usually include a chemical stimulus to increase Ca^{2+} concentrations in the injected oocytes. Examples are ionomycin (Rho *et al.* 1998*a*) or Ca^{2+} ionophore A23187 (Kolbe & Holtz 2000). In the case of pig embryos, an electrical stimulus is more widely employed to induce activation (Lee *et al.* 2003, Lee & Yang 2004, Matsurani *et al.* 2014). These treatments induce a single Ca^{2+} peak in the oocyte, causing a temporary inactivation of MPF that leads to the release from the meiotic arrest. However, it is not enough to induce pronuclei formation, since the inactive state of this factor needs to be maintained to allow complete activation (Kubiak *et al.* 1993, Susko-Parrish *et al.* 1994). For this reason, activation protocols for domestic species combine the use of physical or chemical Ca^{2+} release inducers with an inhibitor of MPF and/or MAPK activities. The most widely used compounds are cycloheximide (CHX), a general inhibitor of protein synthesis (Baliga *et al.* 1969) and 6-dimethylaminopurine (6-DMAP), a protein kinase phosphorylation inhibitor (Szöllösi *et al.* 1993). Both treatments are capable of giving rise to acceptable blastocyst rates in the cow (Suttner *et al.* 2000, Oikawa *et al.* 2005, Bevacqua *et al.* 2010), which explains their extensive use for *in vitro* studies. However, only one newborn has been produced with 6-DMAP (Oikawa *et al.* 2005), while no births with CHX have been reported. Therefore, more specific activation treatments have been proposed, such as the use of a Ca^{2+} ionophore followed by dehydroleucodine (Vichera *et al.* 2010), roscovitine (Fernandes *et al.* 2014) and anisomycin (Arias *et al.* 2016), which were able to produce blastocysts. An important methodological aspect to consider prior to performing ICSI is how the activating agents affect 2PB extrusion. For example, the use of 6-DMAP immediately after Ca^{2+} ionophore inhibits 2PB extrusion (Rho *et al.* 1998*b*), a mistake commonly observed even in current ICSI studies. For this reason, the activation protocol for ICSI embryos must include a window of 3 h between Ca^{2+} ionophore and incubation with 6-DMAP (Ock *et al.* 2003). In contrast, when dehydroleucodine, roscovitine, CHX or anisomycin are used immediately after Ca^{2+} ionophore, 2PB extrusion occurs in most of the oocytes treated (Canel *et al.* 2010,

Table 1 Reported live born offspring after ICSI in bovine, sheep, goat, pig and horse.

Species	Treatment	Live born	Observations	References
Bovine	Ethanol	10	Cell sorted sperm heads	Hamano *et al*. (1999)
	Piezo	3		Wei & Fukui (2002)
	Piezo/ethanol	9 (ethanol)		Oikawa *et al*. (2005)
	Piezo/Io + 6-DMAP	1 (Io + 6-DMAP)		
	Piezo/ethanol	24		Horiuchi *et al*. (2002)
	Piezo/dithiothreitol	1		Galli *et al*. (2003)
Sheep	–	1	Cell sorted	Catt & Rhodes (1995)
	–	2		Gomez *et al*. (1998*a*,*b*)
	–	17	Line hemophilia A	Porada *et al*. (2010)
	–	2		Cochran *et al*. (1998)
Goat	Piezo	2	Fresh sperm	Wang *et al*. (2003)
Pig	–	3	Oocytes *in vivo* matured/centrifuged	Martin (2000)
	Io	1		Kolbe & Holtz (2000)
	$CaCl_2$ activated	13	Cell sorted sperm	Probst & Rath (2003)
	Electrical	3		Nakai *et al*. (2003)
	–	1	Sperm donor was transgenic	Yong *et al*. (2006)
	Piezo. Cysteine	12		Katayama *et al*. (2007)
	–	15 (Tg: live 4 + dead 3)	Recombinase RecA	Garcia-Vasquez *et al*. (2010)
	Electrical. Piezo	62 (tg 8)	BAC	Watanabe *et al*. (2012)
	Electrical. Piezo	6 (tg 2)		Matsunari *et al*. (2014)
Horse	–	2		Cochran *et al*. (1998)
	Piezo	7		Galli *et al*. (2007)
	Piezo	2	Lyophilized sperm	Choi *et al*. (2011)
	Piezo	10	Euthanasia or after death for oocyte donor	Hinrichs *et al*. (2012)

Io, Ca-ionophore; Tg, transgenic.

Arias *et al*. 2016, Suva *et al*. 2016). Additionally, the choice of an activation treatment must not be only based on its capability to produce blastocysts, since it is not necessarily related to the ability to sustain development to term. It is reflected by the outcomes produced by ethanol treatment, which is less efficient than others to produce blastocysts (Bevacqua *et al*. 2010), but has shown the higher birth rates worldwide. In fact, most calves produced by ICSI to date were activated with ethanol (Table 1). Moreover, the use of 6-DMAP has been shown to produce high numbers of ICSI and parthenote embryos with chromosomal abnormalities (De La Fuente & King 1998, Rho *et al*. 1998*b*, Ross *et al*. 2008, Canel *et al*. 2010), explaining in part the frequent pregnancy loss.

Although there are no studies in domestic species, alterations in Ca^{2+} signaling pathways during the activation of murine oocytes have been reported to affect not only early embryonic development, but also gene expression during embryo genome activation and, at the blastocyst stage, implantation and even development to term (Ozil & Huneau 2001, Ozil *et al*. 2006, Rogers *et al*. 2006). Therefore, it is essential to develop activation treatments that better mimic what occurs after regular fertilization. In this sense, Ross *et al*. (2008) proposed an interesting approach. These authors performed the intracytoplasmic injection of *PLCζ1* cRNA into cow oocytes and were able to induce sperm-like Ca^{2+} oscillation patterns, resulting in rates of parthenogenetic development similar to those produced with ionomycin followed by CHX or DMAP. Additionally, embryos activated with *PLCζ1*-cRNA showed lower levels of aneuploidy, which did not differ from those of IVF embryos. Although the use of cRNA is more complex than the routine protocols, these results are promising for their application on ICSI assays.

The second strategy widely employed to facilitate male pronucleus formation is to treat the sperm previous to ICSI, in order to emulate as much as possible how gametes interact in a regular fertilization event. Before *in vivo* fertilization, mammalian sperm cells undergo two physiological events in the female tract: capacitation, that confers the spermatozoon its ability to interact with the oocyte, and acrosome reaction, which consists on the exocytosis of the acrosome content. As a result, the sperm suffer a massive loss of membranes, and after penetrating the zona pellucida, it fuses to the oolemma (Yanagimachi 1994). After fusion, the remaining inner acrosomal membrane is also disrupted, allowing the direct interaction of both sperm and oocyte cytoplasm and the release of PLCζ, which triggers oocyte activation (Malcuit *et al*. 2006*b*, Roldan 2006). In contrast, these events are bypassed when performing ICSI, since an intact spermatozoon is directly injected in the cytoplasm of a mature oocyte. Therefore, the complex structure of sperm membranes maintains the sperm nucleus and the ooplasm separated (Sutovsky & Schatten 2000, Morozumi *et al*. 2006, Roldan 2006), which might be a possible cause of the reduced developmental competence of the resulting embryos (Morozumi *et al*. 2006, Yanagimachi 2011, Aguila *et al*. 2017).

As outlined earlier, several sperm treatments prior to ICSI were designed to remove or modified the sperm membranes for facilitating sperm decondensation and pronuclear formation. Among chemical agents, the most widely used for these purposes is dithiothreitol (DTT). Although some studies have reported that DTT increases blastocyst rates (Rho *et al.* 1998*a*, Wei & Fukui 1999, Oikawa *et al.* 2016) and has led to the birth of a viable calf (Galli *et al.* 2003), no positive effect has been observed in other studies (Suttner *et al.* 2000, Arias *et al.* 2014). Moreover, severe damage on sperm DNA was observed after DTT treatment, which ultimately could affect the quality of ICSI embryos (Sekhavati *et al.* 2012). In recent years, many membrane-disrupting agents were tested, such as Triton X-100 (Lee & Yang 2004), sodium hydroxide (Arias *et al.* 2014), dithiobutylamine (Suttirojpattana *et al.* 2016), lysolecithin (Morozumi *et al.* 2006, Zambrano *et al.* 2017) and methyl-β-cyclodextrin (Arias *et al.* 2017). Some of these treatments raised blastocyst rates, but male pronucleus formation was not improved in all cases. Furthermore, some of them were reported to induce a decrease of PLCζ (Zambrano *et al.* 2016) and their effects on development to term have not been evaluated. The location of PLCζ in the sperm is an important issue to be taken into account for ICSI protocol design, since it varies among species. For example, it was found in the equatorial area of bull sperm (Yoon & Fissore 2007), in the post-acrosomal region and the tail of pig sperm (Nakai *et al.* 2011) and in the acrosomal and equatorial regions of the stallion sperm head as well as in the principal piece of the flagellum (Bedford-Guaus *et al.* 2011). Such differences can be a source of variability in the response of sperm to pre-treatments, including the use of the piezo drill

A more physiological approach was the use of reduced glutathione, an endogenous disulfide bond reducer that in combination with heparin induces *in vitro* decondensation of spermatozoa of several species (Reyes *et al.* 1989, 1996, Sanchez-Vazquez *et al.* 1996, 1998, Delgado *et al.* 2001). Sperm treatment with heparin and glutathione prior to ICSI was shown to facilitate sperm decondensation without damaging DNA and to improve embryo development and blastocyst quality after ICSI in the cow (Sekhavati *et al.* 2012, Canel *et al.* 2017). However, since no offspring were reported, these results should be treated with caution.

Finally, the mechanical damage induced by piezo drill on the sperm deserves special attention. During the ICSI procedure, the sperm tail is intentionally damaged before injection, since it was shown to be a critical step for ultimate success (Wei & Fukui 1999). Some researchers consider that it would cause the release of PLCς and other factors within the oocyte cytoplasm after injection, giving rise to the activation cascade (Yanagida *et al.* 2001, Morozumi *et al.* 2006). It is well known that the sole sperm injection stimulus into the cytoplasm of a mature oocyte is sufficient to activate embryo development in horses (Dell'Aquila *et al.* 2001, Tremoleda *et al.* 2003, Lewis *et al.* 2016). It might be due to the great capability of the equine isoform of PLCς to generate Ca^{2+} peaks, combined with the use of piezo drill, which allows the release of PLCς in the ooplasm. Ca^{2+} peaks induced by equine isoform of PLCς were shown to begin earlier and to have a higher frequency than those observed in other species studied (Sato *et al.* 2013). Therefore, the localization and strength of equine PLCς may explain, at least in part, the major repeatability achieved for ICSI in horses by the use of piezo drill (Galli *et al.* 2002, Choi *et al.* 2003), in contrast to other domestic species, specially the bovine (Katayose *et al.* 1999, Horiuch *et al.* 2002, Wang *et al.* 2003, Devito *et al.* 2010). Nonetheless, it is important to highlight that most new born calves produced by ICSI have been subjected to piezo drill, in combination with an activation treatment (Table 1).

Coincident with the observations of Wei and Fukui (1999), our experience led us to hypothesize that a big part of the variability of ICSI outcomes in domestic species is due to the different responses of males to sperm pre-treatments. Thus, greater knowledge of the mechanisms governing the early events of fertilization is needed to determine the exact combination of sperm pre-treatments and activation protocols required for successful ICSI in domestic species, particularly in the bovine.

ICSI-mediated gene transfer (ICSI-MGT)

The technique of ICSI-MGT is based on the fact that transgenes may spontaneously attach to the external sperm membrane, and then be passively transported into the cytoplasm of a mature oocyte when the spermatozoon is introduced by ICSI. In this way, integration of transgenes is possible during early stages of pronuclei formation (Perry *et al.* 1999). Some benefits associated with ICSI-MGT are that it avoids the epigenetic failures induced by SCNT (Rideout *et al.* 2001) and the high rates of oocyte lysis provoked by pronucleus microinjection. In our laboratory, cow, sheep, horse, pig and domestic cat *GFP* (green fluorescent protein) expressing embryos have been produced by ICSI-MGT (Pereyra Bonnet *et al.* 2008, 2011). Moreover, by the use of improved activation treatments, rates of *GFP*-expressing cow blastocysts exceeded 80% after ICSI-MGT (Bevacqua *et al.* 2010). Although several researchers reported experiments of ICSI-MGT in the cow (Canel *et al.* 2017), ewe (Gou *et al.* 2002), goat (Shadanloo *et al.* 2009), horse (Zaniboni *et al.* 2013) and monkeys (Chan *et al.* 2000), most of them only observed cytoplasmic expression of the transgenes, without giving evidence of their integration into the genome, or the birth of transgenic live animals. In contrast, several transgenic pigs have been successfully generated using this technology (Kurome *et al.* 2006, Yong *et al.* 2006,

Umeyama *et al.* 2012, Matsunari *et al.* 2014). In conclusion, ICSI-MGT in farm animal has only produced repeatable results in pigs, wherein several transgenic offspring have resulted. However, interest in producing transgenic animals by ICSI-MGT has decreased with the advent of new tools of gene edition (CRISPR-Cas9 and TALEN system), which are technically simpler (Hai *et al.* 2014, Whitworth *et al.* 2014, Proudfoot *et al.* 2015, Wang *et al.* 2015, Bevacqua *et al.* 2016).

In our laboratory, an alternative application of ICSI-MGT has been proposed. The high lipid content of cow oocytes impedes the visualization of pronuclei after fertilization. Since ICSI in cattle must be followed by artificial activation, ICSI embryos (which are products of proper sperm decondensation) need to be distinguished from those produced merely by artificial activation. To do this, sperm can be subjected to a brief incubation with pCX-*EGFP* before ICSI. This plasmid contains the gene coding for green fluorescent protein (GFP) under the control of a promoter that is constitutively expressed at early stages of embryo development (Ikawa *et al.* 1995). Such expression can be simply detected by observation of embryos under UV light at day 4 of *in vitro* culture. A previous report from our group (Bevacqua *et al.* 2010) showed that all *GFP*-expressing embryos had successfully undergone pronuclei formation. By contrast, more than 50–100% of embryos without *GFP* expression showed a condensed sperm head inside them, depending on the activation treatment employed. These results reflect a strong association between pCX-*EGFP* expression and sperm head decondensation after ICSI. Therefore, the joint injection of pCX-*EGFP* with sperm, and the subsequent evaluation of *GFP* expression can be used as an indicator of efficient sperm decondensation, as was done by Canel *et al.* (2017). In addition, this method might be easily adapted to other domestic species whose oocytes present similar or even greater lipid content, since sperm–plasmid incubation previous to ICSI also produces *GFP*-expressing embryos (Pereyra Bonnet *et al.* 2008).

Clinical applications for ICSI: a success in horses

Breeding selection in horses is usually based on their sporting performance, beauty or body conformation, rather than reproductive abilities, as is the case for other farm animals. Consequently, subfertility and infertility problems are unintentionally conserved in donor mares and stallions. Several conditions like chronic uterine diseases, endometritis, cervical lacerations and other serious physical injuries in the female reproductive tract frequently reduce or restrict the chances of mares to conceive a pregnancy (Foss *et al.* 2013, Rader *et al.* 2016). As well, some stallions that show good performance are sub-fertile or their sperm supplies are limited, since they are castrated before showing valuable genetic characteristics or die unexpectedly. For these reasons, the advent of new reproductive technologies inevitably led to the inclusion of an *in vitro* embryo production system in horse reproductive programs. In particular, the ICSI technique gained importance in this species since a consistent IVF protocol has not been yet developed, possibly due to an incomplete capacitation of the stallion spermatozoa, that apparently disables them to penetrate the zona pellucida *in vitro* (Leemans *et al.* 2016). Unfortunately, in spite of numerous attempts to make conventional IVF successful for horses, outcomes continue to be disappointing.

After the first report of a pregnancy derived from an *in vitro*-matured oocyte fertilized using ICSI (Squires *et al.* 1996), the encouraging outcome was followed by a period of variable results. The introduction of the piezo drill and modifications in the culture media allowed an improvement in cleavage rates and repeatability of ICSI protocols (Choi *et al.* 2002, 2004, Galli *et al.* 2002). However, reported blastocyst rates still vary from 0 to 42% depending on mare age, follicle stage, oocyte quality and fertility of the stallion (Tremoleda *et al.* 2003, Hinrichs *et al.* 2012, 2013, Foss *et al.* 2013, Choi *et al.* 2016, Rader *et al.* 2016). With the use of these technologies, pregnancy rates after embryo transfer are usually high, varying from 50 to 80% (Hinrichs 2013, Galli *et al.* 2014).

Additional advantages for ICSI are seen in horses. For example, oocytes can be placed at room temperature in commercial embryo-holding media for 18–24 h, so *in vitro* maturation can be delayed allowing a flexible work schedule and simplifying the transport of immature oocytes from the farm to the laboratory (Choi *et al.* 2006, Foss *et al.* 2013, Martino *et al.* 2014, Carnevale 2016, Dini *et al.* 2016). Although the equine is a seasonal species (long day breeders), ICSI can be performed at any time of the year and at any stage of the reproductive cycle, as long as there are follicles present in the ovaries. It avoids interference with training or sporting activities of the donor mares, which is critical for a species of commercial interest. Additionally, in contrast to other species like pigs, horse ICSI embryos can be successfully cryopreserved for later transfer (Galli *et al.* 2002, Hinrichs 2013).

Another interesting alternative for the use of ICSI is when an unfortunate event like accident or illness results in the death or euthanasia of a valuable mare. The application of this technique might offer the chance of getting offspring by recovery of oocytes within 7 h of death (Ribeiro *et al.* 2008, Carnevale 2016). The subsequent ICSI performance using sperm from a desired stallion can result in valuable embryos that can be transferred to a recipient mare or cryopreserved for future transfer (Carnevale *et al.* 2003, Hinrichs *et al.* 2012).

Nowadays, the horse remains at the forefront of the ICSI technique over other domestic species, showing an exponential increase in the use of this technology

in the last two decades. Viable embryos are routinely produced from donor mares in a consistent and repeatable manner, by the combination of transvaginal aspiration followed by ICSI and *in vitro* culture of embryos to the blastocyst stage. Moreover, embryos are obtained using small pieces of frozen semen straws or even from lyophilized sperm (Choi *et al.* 2011). Currently, perspectives are focused on the conservation of female gametes by vitrification. Blastocyst rates after vitrification of immature oocytes have reached values of 10% (Siqueira Canesin *et al.* 2017), showing great potential for female genetic preservation, that might also give rise to new alternatives for genetic rescue of wild equids (Smits *et al.* 2012*b*). However, more research is needed to improve its efficiency and also to unveil the mysteries of the IVF technique in horses. Such discovery would mean a resounding improvement for the horse industry.

A promising technique for endangered and nontraditional species

Small populations of endangered species have a lack of genetic diversity increasing the chance of inbreeding and homozygosis (Roldan *et al.* 2006). This reduces the adaptation capacity and increases the risk of inherited diseases, congenital defects and decreases fertility (Comizzoli *et al.* 2000). In many cases, these animals have reduced sperm quality, which limits regular reproduction or the use of ARTs like AI or IVF (Howard *et al.* 1993, Koester *et al.* 2015), ICSI can be used in cases. Additionally, it is possible to produce offspring from gametes of deceased animals (Fernandez-Gonzalez *et al.* 2015) or improve the fertility of poor sperm (Choi *et al.* 2016) or oocyte quality (Jimenez-Macedo *et al.* 2007, Catala *et al.* 2012, Ohlweiler *et al.* 2013). This technique allows the selection of morphologically normal spermatozoa, even from samples containing a large proportion of teratozoospermic sperm (Penfold *et al.* 2003), which are frequently observed in zoo inbred species. In camelids, where sperm freezing and thawing protocols are not efficient, ICSI might be an option to achieve *in vitro*-produced embryo (Sansinena *et al.* 2007, Conde *et al.* 2008).

Concerning to felids, ICSI in the domestic cat has been a valuable model to develop this technology for their wild counterparts (Moro *et al.* 2014). Some researchers have reported that artificial activation is necessary to restart the oocyte cell cycle after ICSI (Bogliolo *et al.* 2001, Comizzoli *et al.* 2006), while others observed embryo development without the need of any type of activation treatment (Pope *et al.* 1998, Penfold *et al.* 2003, Moro *et al.* 2014). This difference may be correlated with the concentration of PLCζ, which was shown to vary among males (Villaverde *et al.* 2013). Since the first cat was produced by ICSI using fresh semen and *in vivo*-matured oocytes (Pope *et al.* 1998, Gomez *et al.* 2000), kittens have also been produced with the use of frozen semen (Gomez *et al.* 2003, Tharasanit *et al.* 2012). However, there are few reports of blastocysts production by *in vitro* maturation of oocytes by ICSI in wild felids.

In wild or endangered species, oocytes are a limiting factor. Thus, interspecific ICSI can be used to evaluate the fertilizing capability of spermatozoa from exotic species using *in vitro*-matured oocytes from domestic animals. In our laboratory, good rates of ICSI blastocysts were produced after injecting cheetah and leopard spermatozoa into domestic cat oocytes, without any activation treatment (Moro *et al.* 2014). Also Kaneko *et al.* (2014) used mouse oocytes to evaluate freeze-dried sperm samples from the chimpanzee, giraffe, jaguar, weasel and the long-haired rat.

Finally, ICSI allows the reproduction of wild animals that are separated by space (natural habitat and zoos) and time (cryobanking), even when sperm are poorly cryopreserved or in low number.

Final considerations

Nowadays, the low repeatability and the high complexity of ICSI technique in domestic species have restricted this technology to the production of elite horses. It is expected that the use of ICSI would contribute to preserve the genetic diversity of endangered mammals, especially for those species that are closely related to domestic ones, for which ICSI has shown promising results. Currently, the ICSI technique is an unlimited source of information regarding the fertilization process. It offers a great potential for clarifying mechanistic differences among mammalian species, with high impact perspectives in both basic and applied research fields.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements

The authors thank to Elizabeth Crichton for her assistance with English corrections and to Dr Lucia Moro and Dr Federico Pereyra-Bonnet for providing images for this review.

References

Aguila L, Felmer R, Arias ME, Navarrete F, Martin-Hidalgo D, Lee HC, Visconti P & Fissore R 2017 Defective sperm head decondensation undermines the success of ICSI in the bovine. *Reproduction* **154** 207–218. (https://doi.org/10.1530/REP-17-0270)

Alberio R, Zakhartchenko V, Motlik J & Wolf E 2001 Mammalian oocyte activation: lessons from the sperm and implications for nuclear transfer. *International Journal of Developmental Biology* **45** 797–809.

Arias ME, Sanchez R, Risopatron J, Perez L & Felmer R 2014 Effect of sperm pretreatment with sodium hydroxide and dithiothreitol on the efficiency of bovine intracytoplasmic sperm injection. *Reproduction, Fertility and Development* **26** 847–854. (https://doi.org/10.1071/RD13009)

Arias ME, Risopatron J, Sanchez R & Felmer R 2015 Intracytoplasmic sperm injection affects embryo developmental potential and gene expression in cattle. *Reproductive Biology* **15** 34–41. (https://doi.org/10.1016/j.repbio.2014.11.001)

Arias ME, Sanchez R & Felmer R 2016 Effect of anisomycin, a protein synthesis inhibitor, on the in vitro developmental potential, ploidy and embryo quality of bovine ICSI embryos. *Zygote* **24** 724–732. (https://doi.org/10.1017/S0967199416000034)

Arias ME, Sanchez-Villalba E, Delgado A & Felmer R 2017 Effect of transfection and co-incubation of bovine sperm with exogenous DNA on sperm quality and functional parameters for its use in sperm-mediated gene transfer. *Zygote* **25** 85–97. (https://doi.org/10.1017/S096719941600037X)

Baliga BS, Pronczuk AW & Munro HN 1969 Mechanism of cycloheximide inhibition of protein synthesis in a cell-free system prepared from rat liver. *Journal of Biological Chemistry* **244** 4480–4489.

Bedford SJ, Kurokawa M, Hinrichs K & Fissore RA 2003 Intracellular calcium oscillations and activation in horse oocytes injected with stallion sperm extracts or spermatozoa. *Reproduction* **126** 489–499. (https://doi.org/10.1530/rep.0.1260489)

Bedford SJ, Kurokawa M, Hinrichs K & Fissore RA 2004 Patterns of intracellular calcium oscillations in horse oocytes fertilized by intracytoplasmic sperm injection: possible explanations for the low success of this assisted reproduction technique in the horse. *Biology of Reproduction* **70** 936–944. (https://doi.org/10.1095/biolreprod.103.021485)

Bedford-Guaus SJ, McPartlin LA, Xie J, Westmiller SL, Buffone MG & Roberson MS 2011 Molecular cloning and characterization of phospholipase C zeta in equine sperm and testis reveals species-specific differences in expression of catalytically active protein. *Biology of Reproduction* **85** 78–88. (https://doi.org/10.1095/biolreprod.110.089466)

Bevacqua RJ, Pereyra-Bonnet F, Fernandez-Martin R & Salamone DF 2010 High rates of bovine blastocyst development after ICSI-mediated gene transfer assisted by chemical activation. *Theriogenology* **74** 922–931. (https://doi.org/10.1016/j.theriogenology.2010.04.017)

Bevacqua RJ, Fernandez-Martin R, Savy V, Canel NG, Gismondi MI, Kues WA, Carlson DF, Fahrenkrug SC, Niemann H, Taboga OA *et al*. 2016 Efficient edition of the bovine PRNP prion gene in somatic cells and IVF embryos using the CRISPR/Cas9 system. *Theriogenology* **86** 1886–1896. (https://doi.org/10.1016/j.theriogenology.2016.06.010)

Bogliolo L, Leoni G, Ledda S, Naitana S, Zedda M, Carluccio A & Pau S 2001 Intracytoplasmic sperm injection of in vitro matured oocytes of domestic cats with frozen-thawed epididymal spermatozoa. *Theriogenology* **56** 955–967. (https://doi.org/10.1016/S0093-691X(01)00621-5)

Canel N, Bevacqua R, Fernandez-Martin R & Salamone DF 2010 Activation with ionomycin followed by dehydroleucodine and cytochalasin B for the production of parthenogenetic and cloned bovine embryos. *Cell Reprogram* **12** 491–499. (https://doi.org/10.1089/cell.2009.0109)

Canel NG, Bevacqua RJ, Hiriart MI, Rabelo NC, de Almeida Camargo LS, Romanato M, de Calvo LP & Salamone DF 2017 Sperm pretreatment with heparin and L-glutathione, sex-sorting, and double cryopreservation to improve intracytoplasmic sperm injection in bovine. *Theriogenology* **93** 62–70. (https://doi.org/10.1016/j.theriogenology.2016.12.018)

Carnevale EM 2016 Advances in collection, transport and maturation of equine oocytes for assisted reproductive techniques. *Veterinary Clinics of North America: Equine Practice* **32** 379–399. (https://doi.org/10.1016/j.cveq.2016.07.002)

Carnevale EM, Maclellan LJ & Coutinho da Silva MA 2003 Pregnancies attained after collection and transfer of oocytes from ovaries of five euthanized mares. *Journal of the American Veterinary Medical Association* **222** 60–62. (https://doi.org/10.2460/javma.2003.222.60)

Casas I, Sancho S, Briz M, Pinart E, Bussalleu E, Yeste M & Bonet S 2009 Freezability prediction of boar ejaculates assessed by functional sperm parameters and sperm proteins. *Theriogenology* **15** 930–948. (https://doi.org/10.1016/j.theriogenology.2009.07.001).

Catala MG, Izquierdo D, Rodriguez-Prado M, Hammami S & Paramio MT 2012 Effect of oocyte quality on blastocyst development after in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) in a sheep model. *Fertility and Sterility* **97** 1004–1008. (https://doi.org/10.1016/j.fertnstert.2011.12.043)

CattJWRhodesSL 1995 Comparative intracytoplasmic sperm injection (ICSI) in human and domestic species. *Reproduction, Fertility and Development* **7** 161–167.

Catt SL, Catt JW, Gomez MC, Maxwell WM & Evans G 1996 Birth of a male lamb derived from an in vitro matured oocyte fertilized by intracytoplasmic injection of a single presumptive male sperm. *Veterinary Record* **16** 494–495. (https://doi.org/10.1071/RD9950161)

Chan AW, Luetjens CM, Dominko T, Ramalho-Santos J, Simerly CR, Hewitson L & Schatten G 2000 Foreign DNA transmission by ICSI: injection of spermatozoa bound with exogenous DNA results in embryonic GFP expression and live rhesus monkey births. *Molecular Human Reproduction* **6** 26–33. (https://doi.org/10.1093/molehr/6.1.26)

Choi YH, Love CC, Love LB, Varner DD, Brinsko S & Hinrichs K 2002 Developmental competence in vivo and in vitro of in vitro-matured equine oocytes fertilized by intracytoplasmic sperm injection with fresh or frozen-thawed spermatozoa. *Reproduction* **123** 455–465. (https://doi.org/10.1530/rep.0.1230455)

Choi YH, Love CC, Varner DD, Love LB & Hinrichs K 2003 Effects of gas conditions, time of medium change, and ratio of medium to embryo on in vitro development of horse oocytes fertilized by intracytoplasmic sperm injection. *Theriogenology* **59** 1219–1229. (https://doi.org/10.1016/S0093-691X(02)01164-0)

Choi YH, Roasa LM, Love CC, Varner DD, Brinsko SP & Hinrichs K 2004 Blastocyst formation rates in vivo and in vitro of in vitro-matured equine oocytes fertilized by intracytoplasmic sperm injection. *Biology of Reproduction* **70** 1231–1238. (https://doi.org/10.1095/biolreprod.103.023903)

Choi YH, Love LB, Varner DD & Hinrichs K 2006 Holding immature equine oocytes in the absence of meiotic inhibitors: effect on germinal vesicle chromatin and blastocyst development after intracytoplasmic sperm injection. *Theriogenology* **66** 955–963. (https://doi.org/10.1016/j.theriogenology.2006.01.064)

Choi YH, Varner DD, Love CC, Hartman DL & Hinrichs K 2011 Production of live foals via intracytoplasmic injection of lyophilized sperm and sperm extract in the horse. *Reproduction* **142** 529–538. (https://doi.org/10.1530/REP-11-0145)

Choi YH, Velez IC, Macias-Garcia B, Riera FL, Ballard CS & Hinrichs K 2016 Effect of clinically-related factors on in vitro blastocyst development after equine ICSI *Theriogenology* **85** 1289–1296. (https://doi.org/10.1016/j.theriogenology.2015.12.015)

Cochran R, Meintjes M, Reggio B, Hylan D, Carter J, Pinto C, Paccamonti D & Godke RA 1998 Live foals produced from sperm-injected oocytes derived from pregnant mares. *Journal of Equine Veterinary Science* **18** 736–740. (https://doi.org/10.1016/S0737-0806(98)80504-2)

Chung JT, Keefer CL & Downey BR 2000 Activation of bovine oocytes following intracytoplasmic sperm injection (ICSI). *Theriogenology* **53** 1273–1284. (https://doi.org/10.1016/S0093-691X(00)00271-5)

Comizzoli P, Mermillod P & Mauget R 2000 Reproductive biotechnologies for endangered mammalian species. *Reproduction Nutrition Development* **40** 493–504. (https://doi.org/10.1016/j.anireprosci.2006.07.002)

Comizzoli P, Wildt DE & Pukazhenthi BS 2006 In vitro development of domestic cat embryos following intra-cytoplasmic sperm injection with testicular spermatozoa. *Theriogenology* **66** 1659–1663. (https://doi.org/10.1016/j.theriogenology.2006.01.038)

Conde PA, Herrera C, Trasorras VL, Giuliano SM, Director A, Miragaya MH, Chaves MG, Sarchi MI, Stivale D, Quintans C *et al*. 2008 In vitro production of llama (Lama glama) embryos by IVF and ICSI with fresh semen. *Animal Reproduction Science* **109** 298–308. (https://doi.org/10.1016/j.anireprosci.2007.10.004)

Coy P & Romar R 2002 In vitro production of pig embryos: a point of view. *Reproduction, Fertility and Development* **14** 275–286. (https://doi.org/10.1071/RD01102)

De La Fuente R & King WA 1998 Developmental consequences of karyokinesis without cytokinesis during the first mitotic cell cycle of bovine parthenotes. *Biology of Reproduction* **58** 952–962. (https://doi.org/10.1095/biolreprod58.4.952)

Delgado NM, Flores-Alonso JC, Rodriguez-Hernandez HM, Merchant-Larios H & Reyes R 2001 Heparin and glutathione II: correlation between decondensation of bull sperm cells and its nucleons. *Archives of Andrology Journal* **47** 47–58. (https://doi.org/10.1080/01485010152104008)

Dell'Aquila ME, Masterson M, Maritato F & Hinrichs K 2001 Influence of oocyte collection technique on initial chromatin configuration, meiotic competence, and male pronucleus formation after intracytoplasmic sperm injection (ICSI) of equine oocytes. *Molecular Reproduction and Development* **60** 79–88. (https://doi.org/10.17221/8237-cjas)

Devito LG, Fernandes CB, Blanco IDP, Tsuribe PM & Landim-Alvarenga FC 2010 Use of a piezo drill for intracytoplasmic sperm injection into cattle oocytes activated with ionomycin associated with roscovitine. *Reproduction in Domestic Animals* **45** 654–658. (https://doi.org/10.1111/j.1439-0531.2008.01323.x)

Devroey P & Van Steirteghem A 2004 A review of ten years experience of ICSI. *Human Reproduction Update* **10** 19–28. (https://doi.org/10.1093/humupd/dmh004)

Dini P, Bogado Pascottini O, Ducheyne K, Hostens M & Daels P 2016 Holding equine oocytes in a commercial embryo-holding medium: new perspective on holding temperature and maturation time. *Theriogenology* **86** 1361–1368. (https://doi.org/10.1016/j.theriogenology.2016.04.079)

Dozortsev D, Rybouchkin A, De Sutter P & Dhont M 1995 Sperm plasma membrane damage prior to intracytoplasmic sperm injection: a necessary condition for sperm nucleus decondensation. *Human Reproduction* **10** 2960–2964. (https://doi.org/10.1093/oxfordjournals.humrep.a135829)

Ducibella T, Huneau D, Angelichio E, Xu Z, Schultz RM, Kopf GS, Fissore R, Madoux S & Ozil JP 2002 Egg-to-embryo transition is driven by differential responses to Ca(2+) oscillation number. *Developmental Biology* **250** 280–291. (https://doi.org/10.1016/s0012-1606(02)90788-8)

Fernandes CB, Devito LG, Martins LR, Blanco ID, de Lima Neto JF, Tsuribe PM, Gonçalves CG & da Cruz Landim-Alvarenga F 2014 Artificial activation of bovine and equine oocytes with cycloheximide, roscovitine, strontium, or 6-dimethylaminopurine in low or high calcium concentrations. *Zygote* **22** 387–394. (https://doi.org/10.1017/S0967199412000627)

Fernandez-Gonzalez L, Hribal R, Stagegaard J, Zahmel J & Jewgenow K 2015 Production of lion (Panthera leo) blastocysts after in vitro maturation of oocytes and intracytoplasmic sperm injection *Theriogenology* **83** 995–999. (https://doi.org/10.1016/j.theriogenology.2014.11.037)

Foss R, Ortis H & Hinrichs K 2013 Effect of potential oocyte transport protocols on blastocyst rates after intracytoplasmic sperm injection in the horse. *Equine Veterinary Journal* **45** 39–43. (https://doi.org/10.1111/evj.12159)

Fujinami N, Hosoi Y, Kato H, Matsumoto K, Saeki K & Iritani A 2004 Activation with ethanol improves embryo development of ICSI-derived oocytes by regulation of kinetics of MPF activity. *Journal of Reproduction and Development* **50** 171–178. (https://doi.org/10.1262/jrd.50.171)

Galli C, Crotti G, Turini P, Duchi R, Mari G, Zavaglia G, Duchamp G, Daels P & Lazzari G 2002 Frozen–thawed embryos produced by Ovum Pick Up of immature oocytes and ICSI are capable to establish pregnancies in the horse. *Theriogenology* **58** 705–708. (https://doi.org/10.1016/S0093-691X(02)00771-9)

Galli C, Vassiliev I, Lagutina I, Galli A & Lazzari G 2003 Bovine embryo development following ICSI: effect of activation, sperm capacitation and pre-treatment with dithiothreitol. *Theriogenology* **60** 1467–1480. (https://doi.org/10.1016/S0093-691X(03)00133-X)

Galli C, Colleoni S, Duchi R, Lagutina I & Lazzari G 2007 Developmental competence of equine oocytes and embryos obtained by in vitro procedures ranging from in vitro maturation and ICSI to embryo culture, cryopreservation and somatic cell nuclear transfer. *Animal Reproduction Science* **98** 39–55. (https://doi.org/10.1016/j.anireprosci.2006.10.011)

Galli C, Duchi R, Colleoni S, Lagutina I & Lazzari G 2014 Ovum pick up, intracytoplasmic sperm injection and somatic cell nuclear transfer in cattle, buffalo and horses: from the research laboratory to clinical practice. *Theriogenology* **81** 138–151. (https://doi.org/10.1016/j.theriogenology.2013.09.008)

Garcia-Rosello E, Garcia-Mengual E, Coy P, Alfonso J & Silvestre MA 2009 Intracytoplasmic sperm injection in livestock species: an update. *Reproduction in Domestic Animals* **44** 143–151. (https://doi.org/10.1111/j.1439-0531.2007.01018.x)

Garcia-Vazquez FA, Ruiz S, Matas C, Izquierdo-Rico MJ, Grullon LA, De Ondiz A, Vieira L, Aviles-Lopez K, Gutierrez-Adan A & Gadea J 2010 Production of transgenic piglets using ICSI-sperm-mediated gene transfer in combination with recombinase RecA. *Reproduction* **140** 259–272. (https://doi.org/10.1530/REP-10-0129)

Gomez MC, Catt JW, Gillan L, Evans G & Maxwell WM 1997 Effect of culture, incubation and acrosome reaction of fresh and frozen-thawed ram spermatozoa for in vitro fertilization and intracytoplasmic sperm injection. *Reproduction, Fertility and Development* **9** 665–673. (https://doi.org/10.1071/r96122)

Gomez MC, Catt JW, Evans G & Maxwell WM 1998*a* Cleavage, development and competence of sheep embryos fertilized by intracytoplasmic sperm injection and in vitro fertilization. *Theriogenology* **49** 1143–1154. (https://doi.org/10.1016/S0093-691X(98)00062-4)

Gomez MC, Catt JW, Evans G & Maxwell WM 1998*b* Sheep oocyte activation after intracytoplasmic sperm injection (ICSI). *Reproduction, Fertility and Development* **10** 197–205. (https://doi.org/10.1016/S0093-691X(98)00062-4)

Gomez MC, Pope CE, Harris R, Davis A, Mikota S & Dresser BL 2000 Births of kittens produced by intracytoplasmic sperm injection of domestic cat oocytes matured in vitro. *Reproduction, Fertility and Development* **12** 423–433. (https://doi.org/10.1016/j.theriogenology.2012.09.022)

Gomez MC, Pope E, Harris R, Mikota S & Dresser BL 2003 Development of in vitro matured, in vitro fertilized domestic cat embryos following cryopreservation, culture and transfer. *Theriogenology* **60** 239–251. (https://doi.org/10.1016/S0093-691X(03)00004-9)

Goto K, Kinoshita A, Takuma Y & Ogawa K 1990 Fertilization of bovine oocytes by the injection of immobilized, killed spermatozoa. *Veterinary Research* **139** 494–495.

Gou KM, An XR, Tian JH & Chen YF 2002 Sheep transgenic embryos produced by intracytoplasmic sperm injection. *Shi Yan Sheng Wu Xue Bao* **35** 103–108.

Hai T, Teng F, Guo R, Li W & Zhou Q 2014 One-step generation of knockout pigs by zygote injection of CRISPR/Cas system. *Cell Research* **24** 372–375. (https://doi.org/10.1038/cr.2014.11)

Hamano K, Li X, Qian XQ, Funauchi K, Furudate M & Minato Y 1999 Gender preselection in cattle with intracytoplasmically injected, flow cytometrically sorted sperm heads. *Biology of Reproduction* **60** 1194–1197. (https://doi.org/10.1095/biolreprod60.5.1194)

Hinrichs K 2005 Update on equine ICSI and cloning. *Theriogenology* **64** 535–541. (https://doi.org/10.1016/j.theriogenology.2005.05.010)

Hinrichs K 2013 Assisted reproduction techniques in the horse. *Reproduction, Fertility and Development* **25** 80–93. (https://doi.org/10.1071/RD12263)

Hinrichs K, Choi YH, Norris JD, Love LB, Bedford-Guaus SJ, Hartman DL, & Velez IC 2012 Evaluation of foal production following intracytoplasmic sperm injection and blastocyst culture of oocytes from ovaries collected immediately before euthanasia or after death of mares under field conditions. *Journal of the American Veterinary Medical Association* **241** 1070–1074. (https://doi.org/10.2460/javma.241.8.1070)

Hoffmann N, Oldenhof H, Morandini C, Rohn K & Sieme H 2011 Optimal concentrations of cryoprotective agents for semen from stallions that are classified 'good' or 'poor' for freezing. *Animal Reproduction Science* **125** 112–118. (https://doi.org/10.1016/j.anireprosci.2011.03.001).

Horiuchi T & Numabe T 1999 Intracytoplasmic sperm injection (ICSI) in cattle and other domestic animals: problems and improvements in practical use. *Journal of Mammalian Ova Research* **16** 1–9. (https://doi.org/10.1274/jmor.16.1)

Horiuchi T, Emuta C, Yamauchi Y, Oikawa T, Numabe T & Yanagimachi R 2002 Birth of normal calves after intracytoplasmic sperm injection of bovine oocytes: a methodological approach. *Theriogenology* **57** 1013–1024. (https://doi.org/10.1016/S0093-691X(01)00701-4)

Hosoi Y & Iritani A 1993 Rabbit microfertilization. *Molecular Reproduction and Development* **36** 282–284. (https://doi.org/10.1002/mrd.1080360232)

Howard JG, Donoghue AM, Johnston LA & Wildt DE 1993 Zona pellucida filtration of structurally abnormal spermatozoa and reduced fertilization in teratospermic cats. *Biology of Reproduction* **49** 131–139. (https://doi.org/10.1095/biolreprod49.1.131)

Huang T, Kimura Y & Yanagimachi R 1996 The use of piezo micromanipulation for intracytoplasmic sperm injection of human oocytes. *Journal of Assisted Reproduction and Genetics* **13** 320–328. (https://doi.org/10.1007/BF02070146)

Hyakutake T, Suzuki H & Yamamoto S 2015 Effect of viscosity on motion characteristics of bovine sperm. *Journal of Aero Aqua Bio Mechanisms* **4** 63–70. (https://doi.org/10.5226/jabmech.4.63)

Ikawa M, Kominami K, Yoshimura Y, Tanaka K, Nishimune Y & Okabe MA 1995 Rapid and non-invasive selection of transgenic embryos before implantation using green fluorescent protein (GFP). *FEBS Letters* **375** 125–128. (https://doi.org/10.1016/0014-5793(95)01162-8)

Jimenez-Macedo AR, Paramio MT, Anguita B, Morato R, Romaguera R, Mogas T & Izquierdo D 2007 Effect of ICSI and embryo biopsy on embryo development and apoptosis according to oocyte diameter in prepubertal goats. *Theriogenology* **67** 1399–1408. (https://doi.org/10.1016/j.theriogenology.2007.03.003)

Jones KT 2005 Mammalian egg activation: from Ca^{2+} spiking to cell cycle progression. *Reproduction* **130** 813–823. (https://doi.org/10.1530/rep.1.00710)

Kaneko T, Ito H, Sakamoto H, Onuma M & Inoue-Murayama M 2014 Sperm preservation by freeze-drying for the conservation of wild animals. *PLoS ONE* **9** e113381. (https://doi.org/10.1371/journal.pone.0113381)

Katayama M, Rieke A, Cantley T, Murphy C, Dowell L, Sutovsky P & Day BN 2007 Improved fertilization and embryo development resulting in birth of live piglets after intracytoplasmic sperm injection and in vitro culture in a cysteine-supplemented medium. *Theriogenology* **67** 835–847. (https://doi.org/10.1016/j.theriogenology.2006.10.015)

Katayose H, Yanagida K, Shinoki T, Kawahara T, Horiuchi T & Sato A 1999 Efficient injection of bull spermatozoa into oocytes using a Piezo-driven pipette. *Theriogenology* **52** 1215–1224. (https://doi.org/10.1016/s0093-691x(99)00213-7)

Kato Y & Nagao Y 2009 Effect of PVP on sperm capacitation status and embryonic development in cattle. *Theriogenology* **72** 624–635. (https://doi.org/10.1016/j.theriogenology.2009.04.018)

Keskintepe L, Morton PC, Smith SE, Tucker MJ, Simplicio AA & Brackett BG 1997 Caprine blastocyst formation following intracytoplasmic sperm injection and defined culture. *Zygote* **5** 261–265. (https://doi.org/10.1017/s0967199400003701)

KikuchiK OnishiA KashiwazakiN IwamotoM NoguchiJ KanekoH AkitaT Nagai T 2002 Successful piglet production after transfer of blastocysts produced by a modified in vitro system. *Biology of Reproduction* **66** 1033–1041. (https://doi.org/10.1095/biolreprod66.4.1033).

KimuraYYanagimachiR 1995 Intracytoplasmic sperm injection in the mouse. *Biology of Reproduction* **52** 709–720. (https://doi.org/ 10.1095/biolreprod52.4.709)

Kolbe T & Holtz W 2000 Birth of a piglet derived from an oocyte fertilized by intracytoplasmic sperm injection (ICSI). *Animal Reproduction Science* **64** 97–101. (https://doi.org/10.1016/S0378-4320(00)00204-9)

Koester DC, Freeman EW, Brown JL, Wildt DE, Terrell KA, Franklin AD & Crosier AE 2015 Motile sperm output by male cheetahs (Acinonyx jubatus) managed ex situ is influenced by public exposure and number of care-givers. *PLoS ONE* **10** e0135847. (https://doi.org/10.1371/journal.pone.0135847)

Kubiak JZ, Weber M, De Pennart H, Winnston NJ & Maro B 1993 The metaphase arrest in mouse oocytes is controlled through microtubule-dependent destruction of cyclin B in the presence of CSF. *EMBO Journal* **12** 3773–3778.

Kurome M, Ueda H, Tomii R, Naruse K & Nagashima H 2006 Production of transgenic-clone pigs by the combination of ICSI-mediated gene transfer with somatic cell nuclear transfer. *Transgenic Research* **15** 229–240. (https://doi.org/10.1007/s11248-006-0004-5)

Lai L, Sun Q, Wu G, Murphy CN, Kühholzer B, Park KW, Bonk AJ, Day BN & Prather RS 2001 Development of porcine embryos and offspring after intracytoplasmic sperm injection with liposome transfected or non-transfected sperm into in vitro matured oocytes. *Zygote* **9** 39–46. (https://doi.org/10.1017/s0967199401001393)

LazzariG 2002 Frozen–thawed embryos produced by Ovum Pick Up of immature oocytes and ICSI are capable to establish pregnancies in the horse. *Theriogenology* **58** 705–708. (https://doi.org/10.1016/S0093-691X(02)00771-9)

Lee JW & Yang X 2004 Factors affecting fertilization of porcine oocytes following intracytoplasmic injection of sperm. *Molecular Reproduction and Development* **68** 96–102. (https://doi.org/10.1002/mrd.20044)

Lee JW, Tian XC & Yang X 2003 Failure of male pronucleus formation is the major cause of lack of fertilization and embryo development in pig oocytes subjected to intracytoplasmic sperm injection. *Biology of Reproduction* **68** 1341–1347. (https://doi.org/10.1095/biolreprod.102.009001)

Leemans B, Gadella BM, Stout TA, De Schauwer C, Nelis H, Hoogewijs M & Van Soom A 2016 Why doesn't conventional IVF work in the horse? The equine oviduct as a microenvironment for capacitation/fertilization. *Reproduction* **152** R233–R245. (https://doi.org/10.1530/REP-16-0420)

Lewis N, Hinrichs K, Schnauffer K, Morganti M, Mc G & Argo C 2016 Effect of oocyte source and transport time on rates of equine oocytes maturation and cleavage after fertilization by ICSI, with a note on the validation of equine embryo morphological classification. *Clinical Theriogenology* **8** 25–39.

Li XX, Lee DS, Kim KJ, Lee JH, Kim EY, Park JY & Kim MK 2013 Leptin and nonessential amino acids enhance porcine preimplantation embryo development in vitro by intracytoplasmic sperm injection. *Theriogenology* **79** 291–298. (https://doi.org/10.1016/j.theriogenology.2012.08.019)

Lopez-Saucedo J, Paramio-Nieto MT, Fierro R & Piña-Aguilar RE 2012 Intracytoplasmic sperm injection (ICSI) in small ruminants *Animal Reproduction Science* **133** 129–138. (https://doi.org/10.1016/j.anireprosci.2012.07.003)

Malcuit C, Kurokawa M & Fissore RA 2006*a* Calcium oscillations and mammalian egg activation. *Journal of Cellular Physiology* **206** 565–573. (https://doi.org/10.1002/jcp.20471)

Malcuit C, Maserati M, Takahashi Y, Page R & Fissore RA 2006*b* Intracytoplasmic sperm injection in the bovine induces abnormal [Ca2+]i responses and oocyte activation. *Reproduction, Fertility and Development* **18** 39–51. (https://doi.org/10.1071/RD05131)

Markoulaki S, Kurokawa M, Yoon SY, Matson S, Ducibella T & Fissore R 2007 Comparison of Ca2+ and CaMKII responses in IVF and ICSI in the mouse. *Molecular Human Reproduction* 2007 **13** 265–272. (https://doi.org/10.1093/molehr/gal121)

Martin MJ 2000 Development of in vivo-matured porcine oocytes following intracytoplasmic sperm injection. *Biology of Reproduction* **63** 109–112. (https://doi.org/10.1095/biolreprod63.1.109)

Martino NA, Dell'Aquila ME, Filioli Uranio M, Rutigliano L, Nicassio M, Lacalandra GM & Hinrichs K 2014 Effect of holding equine oocytes in meiosis inhibitor-free medium before in vitro maturation and of holding temperature on meiotic suppression and mitochondrial energy/redox potential. *Reproductive Biology and Endocrinology* **12** 99–111. (https://doi.org/10.1186/1477-7827-12-99)

Matsunari H, Kobayashi T, Watanabe M, Umeyama K, Nakano K, Kanai T, Matsuda T, Nagaya M, Hara M, Nakauchi H *et al*. 2014 Transgenic pigs with pancreas-specific expression of green fluorescent protein. *Journal of Reproduction and Development* **60** 230–237. (https://doi.org/10.1262/jrd.2014-006)

Moro LN, Sestelo AJ & Salamone DF 2014 Evaluation of cheetah and leopard spermatozoa developmental capability after interspecific ICSI with domestic cat oocytes. *Reproduction in Domestic Animals* **49** 693–700. (https://doi.org/10.1111/rda.12355)

Morozumi K, Shikano T, Miyazaki S & Yanagimachi R 2006 Simultaneous removal of sperm plasma membrane and acrosome before intracytoplasmic sperm injection improves oocyte activation/embryonic development. *PNAS* **103** 17661–1766. (https://doi.org/10.1073/pnas.0608183103)

Mugnier S, Dell'Aquila ME, Pelaez J, Douet C, Ambruosi B, De Santis T, Lacalandra GM, Lebos C, Sizaret PY, Delaleu B *et al*. 2009 New insights into the mechanisms of fertilization: comparison of the fertilization steps, composition, and structure of the zona pellucida between horses and pigs. *Biology of Reproduction* **81** 856–870. (https://doi.org/10.1095/biolreprod.109.077651)

Nakai M, Kashiwazaki N, Takizawa A, Hayashi Y, Nakatsukasa E, Fuchimoto D, Noguchi J, Kaneko H, Shino M & Kikuchi K 2003 Viable piglets generated from porcine oocytes matured in vitro and fertilized by intracytoplasmic sperm head injection. *Biology of Reproduction* **68** 1003–1008. (https://doi.org/10.1095/biolreprod.102.009506)

Nakai M, Kashiwazaki N, Takizawa A, Maedomari N, Ozawa M, Noguchi J, Kaneko H, Shino M & Kikuchi K 2006 Morphologic changes in boar sperm nuclei with reduced disulfide bonds in electrostimulated porcine oocytes. *Reproduction* **131** 603–611. (https://doi.org/10.1530/rep.1.01001)

Nakai M, Ito J, Sato K, Noguchi J, Kaneko H, Kashiwazaki N & Kikuchi K 2011 Pre-treatment of sperm reduces success of ICSI in the pig. *Reproduction* **142** 285–293. (https://doi.org/10.1530/REP-11-0073)

Nakai M, Ito J, Suzuki SI, Fuchimoto DI, Sembon S, Suzuki M, Noguchi J, Kaneko H, Onishi A, Kashiwazaki N *et al*. 2016*a* Lack of calcium oscillation causes failure of oocyte activation after intracytoplasmic sperm injection in pigs. *Journal of Reproduction and Development* **62** 615–621. (https://doi.org/10.1262/jrd.2016-113)

Nakai M, Suzuki SI, Ito J, Fuchimoto DI, Sembon S, Noguchi J, Onishi A, Kashiwazaki N & Kikuchi K 2016*b* Efficient pig ICSI using Percoll-selected spermatozoa; evidence for the essential role of phospholipase C-ζ in ICSI success. *Journal of Reproduction and Development* **62** 639–643. (https://doi.org/10.1262/jrd.2016-103)

Ock SA, Bhak JS, Balasubramanian S, Lee HJ, Choe SY & Rho GJ 2003 Different activation treatments for successful development of bovine oocytes following intracytoplasmic sperm injection. *Zygote* **11** 69–76. (https://doi.org/10.1017/S0967199403001096)

Ohlweiler LU, Brum DS, Leivas FG, Moyses AB, Ramos RS, Klein N, Mezzalira JC & Mezzalira A 2013 Intracytoplasmic sperm injection improves in vitro embryo production from poor quality bovine oocytes. *Theriogenology* **79** 778–783. (https://doi.org/10.1016/j.theriogenology.2012.12.002)

Oikawa T, Takada N, Kikuchi, T, Numabe T, Takenaka M & Horiuchi T 2005 Evaluation of activation treatments for blastocyst production and birth of viable calves following bovine intracytoplasmic sperm injection. *Animal Reproduction Science* **86** 187–194. (https://doi.org/10.1016/j.anireprosci.2004.07.003)

Oikawa T, Itahashi T & Numabe T 2016 Improved embryo development in Japanese black cattle by in vitro fertilization using ovum pick-up plus intracytoplasmic sperm injection with dithiothreitol. *Journal of Reproduction and Development* **62** 11–16. (https://doi.org/10.1262/jrd.2015-067)

Ozil JP & Huneau D 2001 Activation of rabbit oocytes: the impact of the Ca^2 signal regime on development. *Development* **128** 917–928.

Ozil JP, Banrezes B, Toth S, Pan H & Schultz RM 2006 Ca^2 oscillatory pattern in fertilized mouse eggs affects gene expression and development to term. *Developmental Biology* **300** 534–544. (https://doi.org/10.1016/j.ydbio.2006.08.041)

Palermo G, Joris H, Devroey P & Van Steirteghem AC 1992 Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* **340** 17–18. (https://doi.org/10.1016/0140-6736(92)92425-f)

Penfold LM, Jost L, Evenson DP & Wildt DE 2003 Normospermic versus teratospermic domestic cat sperm chromatin integrity evaluated by flow cytometry and intracytoplasmic sperm injection. *Biology of Reproduction* **69** 1730–1735. (https://doi.org/10.1095/biolreprod.103.016089)

Pereyra Bonnet F, Fernandez-Martin R, Olivera R, Jarazo J, Vichera G, Gibbons A & Salamone D. 2008 A unique method to produce transgenic embryos in ovine, porcine, feline, bovine and equine species. *Reproduction, Fertility and Development* **20** 741–749. (https://doi.org/10.1071/RD07172)

Pereyra Bonnet F, Gibbons A, Cueto M, Sipowicz P, Fernandez-Martin R & Salamone D 2011 Efficiency of sperm-mediated gene transfer in the ovine by laparoscopic insemination, in vitro fertilization and ICSI. *Journal of Reproduction and Development* **57** 188–196. (https://doi.org/10.1262/jrd.10-063A)

Perry AC, Wakayama T, Kishikawa H, Kasai T, Okabe M, Toyoda Y & Yanagimachi R 1999 Mammalian transgenesis by intracytoplasmic sperm injection. *Science* **284** 1180–1183. (https://doi.org/10.1126/science.284.5417.1180)

Pope CE, Johnson CA, McRae MA, Keller GL & Dresser BL 1998 Development of embryos produced by intracytoplasmic sperm injection of cat oocytes. *Animal Reproduction Science* **53** 221–236. (https://doi.org/10.1016/S0378-4320(98)00115-8)

Porada CD, Sanada C, Long CR, Wood JA, Desai J, Frederick N, Millsap L, Bormann C, Menges SL, Hanna C *et al*. 2010 Clinical and molecular characterization of a re-established line of sheep exhibiting hemophilia A. *Journal of Thrombosis and Haemostasis* **8** 276–285. (https://doi.org/10.1111/j.1538-7836.2009.03697.x)

Probst S & Rath D 2003 Production of piglets using intracytoplasmic sperm injection (ICSI) with flowcytometrically sorted boar semen and artificially activated oocytes. *Theriogenology* **59** 961–973. (https://doi.org/10.1016/s0093-691x(02)01135-4)

Proudfoot C, Carlson DF, Huddart R, Long CR, Pryor JH, King TJ, Lillico SG, Mileham AJ, McLaren DG, Whitelaw CB *et al*. 2015 Genome edited sheep and cattle. *Transgenic Research* **24** 147–153. (https://doi.org/10.1007/s11248-014-9832-x).

Rader K, Choi YH & Hinrichs K 2016 Intracytoplasmic sperm injection, embryo culture, and transfer of in vitro-produced blastocysts *Veterinary Clinics of North America: Equine Practice* **32** 401–413. (https://doi.org/10.1016/j.cveq.2016.07.003)

Reyes R, Rosado A, Hernandez O & Delgado NM 1989 Heparin and glutathione: physiological decondensing agents of human sperm nuclei. *Gamete Research* **23** 39–47. (https://doi.org/10.1002/mrd.1120230105)

Reyes R, Sanchez-Vazquez ML, Merchant-Larios H, Rosado A & Delgado NM 1996 Effect of heparin-reduced glutathione on hamster sperm DNA unpacking and nuclear swelling. *Archives of Andrology* **37** 33–45. (https://doi.org/10.3109/01485019608988500)

Ribeiro BI, Love LB, Choi YH & Hinrichs K 2008 Transport of equine ovaries for assisted reproduction. *Animal Reproduction Science* **108** 171–179. (https://doi.org/10.1016/j.anireprosci.2007.08.001)

Rideout WM, Eggan K & Jaenisch R 2001 Nuclear cloning and epigenetic reprogramming of the genome. *Science* **293** 1093–1098. (https://doi.org/10.1126/science.1063206)

Rho GJ, Kawarsky S, Johnson WH, Kochhar K & Betteridge KJ 1998*a* Sperm and oocyte treatments to improve the formation of male and female pronuclei and subsequent development following intracytoplasmic sperm injection into bovine oocytes. *Biology of Reproduction* **59** 918–924. (https://doi.org/10.1095/biolreprod59.4.918)

Rho GJ, Wu B, Kawarsky S, Leibo SP & Betteridge KJ 1998*b* Activation regimens to prepare bovine oocytes for intracytoplasmic sperm injection. *Molecular Reproduction and Development* **50** 485–492. (https://doi.org/10.1002/(SICI)1098-2795(199808)50:4<485::AID-MRD12>3.0.CO;2-1)

Rogers NT, Halet G, Piao Y, Carroll J, Ko MSH & Swann K 2006 The absence of Ca^{2+} signal during mouse egg activation can affect parthenogenetic preimplantation development, gene expression patterns, and blastocyst quality. *Reproduction* **132** 45–57. (https://doi.org/10.1530/rep.1.01059)

Roldan ER 2006 Better intracytoplasmic sperm injection without sperm membranes and acrosome. *PNAS* **103** 17585–17586. (https://doi.org/10.1073/pnas.0608752103)

Roldan ER, Gomendio M, Garde JJ, Espeso G, Ledda S, Berlinguer F, del Olmo A, Soler AJ, Arregui L, Crespo C *et al*. 2006 Inbreeding and reproduction in endangered ungulates: preservation of genetic variation through the Organization of Genetic Resource Banks. *Reproduction in Domestic Animals Supplement* **2** 82–92. (https://doi.org/10.1111/j.1439-0531.2006.00772.x)

Ross PJ, Beyhan Z, Iager AE, Yoon SY, Malcuit C, Schellander K, Fissore RA & Cibelli JB 2008 Parthenogenetic activation of bovine oocytes using bovine and murine phospholipase C zeta. *BMC Developmental Biology* **8** 16–28. (https://doi.org/10.1186/1471-213X-8-16)

Sanchez-VazquezML ReyesR RamirezG Merchant-LariosH RosadoA DelgadoNM1998 DNA unpacking in guinea pig sperm chromatin by heparin and reduced glutathione. *Archives in Andrology* **40** 15–28.

Sansinena MJ, Taylor SA, Taylor PJ, Schmidt EE, Denniston RS & Godke RA 2007 In vitro production of llama (Lama glama) embryos by intracytoplasmic sperm injection: effect of chemical activation treatments and culture conditions. *Animal Reproduction Science* **99** 342–353. (https://doi.org/10.1016/j.anireprosci.2006.05.020)

Sato MS, Yoshitomo M, Mohri T & Miyazaki S 1999 Spatiotemporal analysis of [Ca2+]i rises in mouse eggs after intracytoplasmic sperm injection (ICSI) *Cell Calcium* **26** 49–58. (https://doi.org/10.1054/ceca.1999.0053)

Sato K, Wakai T, Seita Y, Takizawa A, Fissore RA, Ito J & Kashiwazaki N 2013 Molecular characteristics of horse phospholipase C zeta (PLCζ). *Animal Science Journal* **84** 359–368. (https://doi.org/10.1111/asj.12044)

Saunders CM, Larman MG, Parrington J, Cox LJ, Royse J, Blayney LM, Swann K & Lai FA 2002 PLC zeta: a sperm-specific trigger of Ca(2+) oscillations in eggs and embryo development. *Development* **129** 3533–3544.

Sekhavati MH, Shadanloo F, Hosseini MS, Tahmoorespur M, Nasiri MR, Hajian M & Nasr-Esfahani MH 2012 Improved bovine ICSI outcomes by sperm selected after combined heparin-glutathione treatment. *Cell Reprogram* **14** 295–304. (https://doi.org/10.1089/cell.2012.0014)

Shadanloo F, Najafi MH, Hosseini SM, Hajian M, Forouzanfar M, Ghaedi K, Abedi Shirazi A, Ostad-Hosseini S, Ahmadi E, Heidari B & Shams-Esfandabadi N 2009 In vitro developmental competence of ICSI-derived activated ovine embryos. *Theriogenology* **71** 342–348. (https://doi.org/10.1016/j.theriogenology.2008.07.027)

Shirazi A, Derakhshan-Horen M, Pilvarian AA, Ahmadi E, Nazari H & Heidari B 2011 Effect of pre-treatment of ovine sperm on male pronuclear formation and subsequent embryo development following intracytoplasmic sperm injection. *Reproduction in Domestic Animals* **46** 87–94. (https://doi.org/10.1111/j.1439-0531.2010.01593.x)

Simopoulou M, Giannelou P, Bakas P, Gkoles L, Kalampokas T, Pantos K & Koutsilieris M 2016 Making ICSI safer and more effective: a review of the human oocyte and ICSI practice. *In Vivo* **30** 387–400.

Siqueira Canesin H, Gatto Brom-de-Luna J, Choi YH, Ortiz I, Diaw M & Hinrichs K 2017 Blastocyst development after intracytoplasmic sperm injection of equine oocytes vitrified at the germinal-vesicle stage. *Cryobiology* **75** 52–59. (https://doi.org/10.1016/j.cryobiol.2017.02.004)

Smits K, Govaere J, Hoogewijs M, Piepers S & Van Soom A 2012*a* A pilot comparison of laser-assisted vs piezo drill ICSI for the in vitro production of horse embryos. *Reproduction in Domestic Animals* **47** e1–e3. (https://doi.org/10.1111/j.1439-0531.2011.01814.x)

Smits K, Hoogewijs M, Woelders H, Daels P & Van Soom A 2012*b* Breeding or assisted reproduction? Relevance of the horse model applied to the conservation of endangered equids. *Reproduction in Domestic Animals* **47** (Supplement 4) 239–248. (https://doi.org/10.1111/j.1439-0531.2012.02082.x)

Squires EL, Wilson JM, Kato H & Blaszczyk A 1996 A pregnancy after intracytoplasmic sperm injection into equine oocytes matured in vitro *Theriogenology* **45** 306. (https://doi.org/10.1016/0093-691x(96)84779-0)

Stein P & Schultz RM 2012 ICSI in the mouse. *Methods in Enzymology* **476** 251–262. (https://doi.org/10.1016/S0076-6879(10)76014-6)

Stricker SA 1999 Comparative biology of calcium signaling during fertilization and egg activation in animals. *Developmental Biology* **211** 157–176. (https://doi.org/10.1006/dbio.1999.9340)

Susko-Parrish JL, Leibfried-Rutledge ML, Northey DL, Schutzkus V & First NL 1994 Inhibition of protein kinases after an induced calcium transient causes transition of bovine oocytes to embryonic cycles without meiotic completion. *Developmental Biology* **166** 729–739. (https://doi.org/10.1006/dbio.1994.1351)

Sutovsky P & Schatten G 2000 Paternal contributions to the mammalian zygote: fertilization after sperm-egg fusion. *International Review of Cytology* **195** 1–65. (https://doi.org/10.1016/S0074-7696(08)62703-5)

Suttirojpattana T, Somfai T, Matoba S, Nagai T, Parnpai R & Geshi M 2016 Pretreatment of bovine sperm with dithiobutylamine (DTBA) significantly improves embryo development after ICSI. *Journal of Reproduction and Development* **62** 577–585. (https://doi.org/10.1262/jrd.2016-084)

Suttner R, Zakhartchenko V, Stojkovic P, Muller S, Alberio R, Medjugorac I, Brem G, Wolf E & Stojkovic M 2000 Intracytoplasmic sperm injection in bovine: effects of oocyte activation, sperm pretreatment and injection technique. *Theriogenology* **54** 935–948. (https://doi.org/10.1016/S0093-691X(00)00403-9)

Suva M, Canel NG & Salamone D 2016 Haploid activation of bovine oocytes with ionomycin and single or combined activating agents. *Reproduction, Fertility and Development* **28** 225. (https://doi.org/10.1071/RDv28n2Ab188)

Swann K & Lai FA 2016 Egg activation at fertilization by a soluble sperm protein. *Physiological Reviews* **96** 127–149. (https://doi.org/10.1152/physrev.00012.2015)

Szollosi MS, Kubiak JZ, Debey P, de Pennart H, Szollosi D & Maro B 1993 Inhibition of protein kinases by 6-dimethylaminopurine accelerates the transition to interphase in activated mouse oocytes. *Journal of Cell Science* **104** 861–872.

Tesarik J, Sousa M & Testart J 1994 Human oocyte activation after intracytoplasmic sperm injection. *Human Reproduction* **9** 511–518. (https://doi.org/10.1093/oxfordjournals.humrep.a138622)

Tharasanit T, Buarpung S, Manee-In S, Thongkittidilok C, Tiptanavattana N, Comizzoli P & Techakumphu M 2012 Birth of kittens after the transfer of frozen-thawed embryos produced by intracytoplasmic sperm injection with spermatozoa collected from cryopreserved testicular tissue. *Reproduction in Domestic Animals* **47** 305–308. (https://doi.org/10.1111/rda.12072)

Tremoleda JL, Stout TAE, Lagutina I, Lazzari G, Bevers MM, Colenbrander B & Galli C 2003 Effects of in vitro production on horse embryo morphology, cytoskeletal characteristics, and blastocyst capsule formation. *Biology of Reproduction* **69** 1895–1906. (https://doi.org/10.1095/biolreprod.103.018515)

Uehara T & Yanagimachi R 1976 Microsurgical injection of spermatozoa into hamster eggs with subsequent transformation of sperm nuclei into male pronuclei. *Biology of Reproduction* **15** 467–470. (https://doi.org/10.1095/biolreprod15.4.467)

Umeyama K, Saito H, Kurome M, Matsunari H, Watanabe M, Nakauchi H & Nagashima H 2012 Characterization of the ICSI-mediated gene transfer method in the production of transgenic pigs *Molecular Reproduction and Development* **79** 218–228. (https://doi.org/10.1002/mrd.22015)

Vichera G, Alfonso J, Duque CC, Silvestre MA, Pereyra-Bonnet F, Fernandez-Martin R & Salamone D 2010 Chemical activation with a combination of ionomycin and dehydroleucodine for production of parthenogenetic, ICSI and cloned bovine embryos. *Reproduction in Domestic Animals* **45** 306–312. (https://doi.org/10.1111/j.1439-0531.2009.01563.x)

Villaverde AI, Fioratti EG, Fissore RA, He C, Lee HC, Souza FF, Landim-Alvarenga FC & Lopes MD 2013 Identification of phospholipase C zeta in normospermic and teratospermic domestic cat sperm. *Theriogenology* **80** 722–729. (https://doi.org/10.1016/j.theriogenology.2013.06.005)

Wang B, Baldassarre H, Pierson J, Cote F, Rao KM & Karatzas CN 2003 The in vitro and in vivo development of goat embryos produced by intracytoplasmic sperm injection using tail-cut spermatozoa. *Zygote* **11** 219–227. (https://doi.org/10.1017/S0967199403002260)

Wang X, Yu H, Lei A, Zhou J, Zeng W, Zhu H, Dong Z, Niu Y, Shi B, Cai B *et al*. 2015 Generation of gene-modified goats targeting MSTN and FGF5 via zygote injection of CRISPR/Cas9 system. *Scientific Reports* **5** 13878. (https://doi.org/10.1038/srep13878)

Watanabe M, Kurome M, Matsunari H, Nakano K, Umeyema K, Shiota A, Nakauchi H, & Nagashima H 2012 The creation of transgenic pigs expressing human proteins using BAC-derived, full-length genes and intracytoplasmic sperm injection-mediated gene transfer. *Transgenic Research* **21** 605–618. (https://doi.org/10.1007/s11248-011-9561-3)

Wei H & Fukui Y 1999 Effects of bull, sperm type and sperm pretreatment on male pronuclear formation after intracytoplasmic sperm injection in cattle. *Reproduction, Fertility and Development* **11** 59–65. (https://doi.org/10.1071/rd98106)

Wei H & Fukui Y 2002 Births of calves derived from embryos produced by intracytoplasmic sperm injection without exogenous oocyte activation. *Zygote* **10** 149–153. (https://doi.org/10.1017/S0967199402002204)

Whitworth KM, Lee K, Benne JA, Beaton BP, Spate LD, Murphy SL, Samuel MS, Mao J, O'Gorman C, Walters EM *et al*. 2014 Use of the CRISPR/Cas9 system to produce genetically engineered pigs from in vitro-derived oocytes and embryos. *Biology of Reproduction* **91** 78–92. (https://doi.org/10.1095/biolreprod.114.121723)

Yanagida K, Katayose H, Hirata S, Yazawa H, Hayashi S & Sato A 2001 Influence of sperm immobilization on onset of Ca(2+) oscillations after ICSI. *Human Reproduction* **16** 148–152. (https://doi.org/10.1093/humrep/16.1.148)

Yanagimachi R 1994 Mammalian fertilization. In *The Physiology of Reproduction*, 2nd edn., Vol. **1**, pp 189–318. Eds**JD Knobil** &**JD Neill**. New York, NY: Raven Press Ltd.

Yanagimachi R 2011 Problems of sperm fertility: a reproductive biologist's view. *Systems Biology in Reproductive Medicine* **57** 102–114. (https://doi.org/10.3109/19396368.2010.507860)

Yong HY, Hao Y, Lai L, Li R, Murphy CN, Rieke A, Wax D, Samuel M & Prather RS 2006 Production of a transgenic piglet by a sperm injection technique in which no chemical or physical treatments were used for oocytes or sperm. *Molecular Reproduction and Development* **73** 595–599. (https://doi.org/10.1002/mrd.20477)

Yoon SY & Fissore RA 2007 Release of phospholipase C zeta and [Ca2+]i oscillation-inducing activity during mammalian fertilization. *Reproduction* **134** 695–704. (https://doi.org/10.1530/REP-07-0259)

Yoshida N & Perry AC 2007 Piezo-actuated mouse intracytoplasmic sperm injection (ICSI). *Nature Protocols* **2** 296–304. (https://doi.org/10.1038/nprot.2007.7)

Zambrano F, Aguila L, Arias ME, Sanchez R & Felmer R 2016 Improved preimplantation development of bovine ICSI embryos generated with spermatozoa pretreated with membrane-destabilizing agents lysolecithin and Triton X-100. *Theriogenology* **86** 1489–1497. (https://doi.org/10.1016/j.theriogenology.2016.05.007)

Zambrano F, Aguila L, Arias ME, Sanchez R & Felmer R 2017 Effect of sperm pretreatment with glutathione and membrane destabilizing agents lysolecithin and Triton X-100, on the efficiency of bovine intracytoplasmic sperm injection. *Reproduction in Domestic Animals* **52** 305–311. (https://doi.org/10.1111/rda.12906)

Zaniboni A, Merlo B, Zannoni A, Bernardini C, Lavitrano M, Forni M, Mari G & Bacci ML 2013 Expression of fluorescent reporter protein in equine embryos produced through intracytoplasmic sperm injection mediated gene transfer (ICSI-MGT). *Animal Reproduction Science* **137** 53–61. (https://doi.org/10.1016/j.anireprosci.2012.12.010)

Received 14 June 2017
First decision 14 July 2017
Revised manuscript received 21 November 2017
Accepted 1 December 2017