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Past, present and future of ICSI in livestock species

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ABSTRACT

During the past 2 decades, intracytoplasmic sperm injection **(**ICSI) has become a routine technique for clinical applications in humans. The widespread use among domestic species, however, has been limited to horses. In horses, ICSI is used to reproduce elite individuals and, as well as in humans, to mitigate or even circumvent reproductive barriers. Failures in superovulation and conventional *in vitro* fertilization **(**IVF) have been the main reason for the use of this technology in horses. In pigs, ICSI has been successfully used to produce transgenic animals. A series of factors have resulted in implementation of ICSI in pigs: need to use zygotes for numerous technologies, complexity of collecting zygotes surgically, and problems of polyspermy when there is utilization of IVF procedures. Nevertheless, there have been very few additional reports confirming positive results with the use of ICSI in pigs. The ICSI procedure could be important for use in cattle of high genetic value by maximizing semen utilization, as well as for utilization of spermatozoa from prepubertal bulls, by providing the opportunity to shorten the generation interval. When attempting to utilize ICSI in ruminants, there are some biological limitations that need to be overcome if this procedure is going to be efficacious for making genetic improvements in livestock in the future. In this review article, there is an overview and projection of the methodologies and applications that are envisioned for ICSI utilization in these species in the future.

Keywords: ICSI; Domestic Species; Horses; Cattle; Pigs

1. Introduction

Intracytoplasmic sperm injection **(**ICSI) is a micromanipulation technique that allows the injection of one spermatozoon into the ooplasm of a metaphase II oocyte **(**Palermo et al., 1992). In humans, ICSI has been utilized for more than a quarter of a century and has become one of the most widely used assisted reproductive technologies for reproduction in humans. The widespread use of ICSI is the result of the capacity for this procedure to be utilized in addressing many reproductive problems, such as lack of sperm motility and globozoospermia, which are mainly related to male infertility **(**Palermo et al., 1995, 1999; Wallach et al., 1996). Furthermore, ICSI has been utilized to overcome some problems when there is female infertility, such as less-than-optimal quality oocytes and/or when it is only possible to collect a small number of oocytes. Other reproductive problems can be addressed, by utilizing the ICSI procedures, such as premature exocytosis of cortical granules that occurs when utilizing cryopreserved oocytes which have hardening of the zona as a result of utilizing this technique for oocyte preservation **(**Porcu et al., 1997) and perturbations of cortical granule exocytosis that can result when aged oocytes are used when conducting *in vitro* fertilization procedures leading to polyspermy or fertilization failure **(**Schalkoff et al., 1989, Vincent et al.,1990).

In addition, results of several reports indicate that with the utilization of ICSI there is a reduction in the transmission of diseases such as hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV), among others **(**reviewed by Palermo et al., 2017). The use of this technique has also been extended to domestic and non-domesticated species, **(**reviewed by Salamone et al., 2017; Unnikrishnan et al., 2021). Similar to humans, ICSI is an efficacious technique for addressing reproductive problems in horses and mainly to reproduce genetically and/or phenotypically elite individuals. In horses, failures when imposing techniques to induce superovulation and in conventional IVF have been the impetus for the use of ICSI. In cattle, due to the inherent biological characteristics of the sperm, when there is utilization of ICSI, there are associated failures of chromatin decondensation and oocyte activation. Regardless of these issues, increasing the efficacy of ICSI techniques in cattle could facilitate the use this methodology to decrease the generation interval. In pigs, there is the use of ICSI because zygotes are needed for genetic modification, as well as because of the complexity of collecting zygotes surgically or the frequent polyspermy problems when using IVF procedures **(**reviewed by Coy and Romar 2002). For this reason, the focus of

this review article is on analyzing the application of ICSI in livestock species, identifying biological limitations and assessing potential improvements for conducting this technique.

1.1.General technique overview

The ICSI technique is performed using different procedural regimens and subtle variations in utilization among laboratories (Choi et al., 2011; Rodríguez et al., 2019; Lazzari et al., 2020). To perform ICSI, it is essential to use an inverted microscope with a dual-arm micromanipulation system. One of the arms is used for the holding pipette and the other for a beveled and spiked tip injection pipette.

Because sperm size varies among species, injection pipettes of different inner diameters are required, which are usually 9 µm inner diameter pipettes for bulls and pigs and 7 µm for horses. This procedure is typically performed by using the upturned lid of a 150-mm petri dish with 50-µL droplets of oocyte-holding media, frequently Hepes-buffered Tyrode´s medium containing albumin, lactate and pyruvate (TALP-H, Bavister et al., 1977), under mineral oil. Spermatozoa must be placed in another 3-µL droplet of polyvinylpyrrolidone (PVP), as the considerable viscosity of PVP results in a reduction of sperm motility (Hyakutake et al., 2015). The spermatozoon tail is cut, pushing it against the petri dish with the microinjection pipette, to immobilize the gamete. The spermatozoon is subsequently aspirated by the tail keeping the head close to the tip. The pipette is moved into the TALP-H droplet containing the denuded MII oocytes attached to the holding pipette. Both pipettes are placed in aligned opposite sides, and the first polar body must be in the 6 or 12 o'clock position to avoid the spindle area during the time when spermatozoon injection is occurring. Firstly, the injection pipette is pressed against the zona pellucida and subsequently through the oolemma. A subtle aspiration of the ooplasm is necessary to perforate the plasma membrane, producing a change in resistance that rapidly increases the speed and the entry of the ooplasm material into the injection pipette. During this time, the spermatozoon is released into the cytoplasm. The ICSI pipette is gently removed from the ooplasm. In large species, presumptive zygotes are cultured *in vitro* to the blastocyst stage for subsequently conducting non-surgical embryo transfer.

In previous reports there is a description of the surgical transfer of presumptive ICSI-zygotes into the mare (Cochran et al., 1998, McKinnon et al., 2000, Galli et al., 2002; Carnevale et al., 2004) and into the sheep (Lazzari et al., 2000) oviduct as a common practice for the development to the blastocyst stage. Recent improvements in the *in vitro* culture conditions, however, have resulted in this practice being unnecessary (Hinrichs et al., 2005; Choi et al., 2006; Stout, 2020, Lazzari, 2020). Furthermore, there are disadvantages of surgical embryo transfer such as the complexity in conducting the procedure, economic costs, and the detrimental effects on animal welfare (Stout et al., 2006).

1.2. Use of the piezo drill for ICSI

The ICSI technique can also be performed using the piezo drill (PD) system. With this technique, a blunt injection micropipette with piezo-driven micro-vibration capacity is used to penetrate the zona pellucida and rupture the plasma membrane **(**Kimura et al., 1995), resulting in the procedure being easier to conduct. Based on results in some studies this is a more efficacious procedure than other approaches for conducting the ICSI procedures **(**Huang et al., 1996, Wei and Fukui, 2002; Choi et al., 2002; Lazzari et al., 2002; Yoshida and Perry, 2007). This could be extremely useful in some species, such as cattle, in which plasma membranes are a greater resistance barrier to the microinjection pipette, as well as in sheep oocytes, which are more sensitive to micromanipulation with there being greater lysis rates after sperm injection **(**Salamone et al., 2017). It has been suggested that utilization of the PD device could cause sperm membrane integrity disruption with the subsequent release of the equine isoform of phospholipase C (PLCç), a sperm protein that induces oocyte activation **(**Yanagida et al., 2001; Morozumi et al., 2006). The lack of the integrity of the plasma membrane after immobilization with the PD was evident when there was staining with membrane-impermeable compounds in human and pig sperm. Furthermore, in sheep, 46% of sperm had acrosomal disruption when there was use of a PD, facilitating embryo development **(**Anzalone et al., 2016). There is breakdown of the acrosomal membrane before ICSI is performed for oocyte fertilization and embryonic development in several species **(**Kimura and Yanagimachi, 1995; Morozumi et al., 2006; Seita et al., 2009; Zambrano et al., 2016). In humans, results from some studies indicated use of the PD led to an improvement in the formation of pronuclei **(**PN) **(**Hiraoka et al., 2015) and there were

resulting greater pregnancy or live birth rates **(**Yanagida et al.,1998; Takeuchi et al., 2001). The equipment with piezo-devices included is expensive, and effective utilization requires training. The performance of the PD technique is with greater efficacy when liquid mercury is used in the pipette tip; however, this is an environmental pollutant and is neurotoxic. Fluorinert, a perfluorinated oil, has been used to replace the mercury **(**Smits et al., 2018), however, this compound is also a toxic and environmental pollutant.

In horses, the simple sperm injection into a matured oocyte results in activation and development of the embryo **(**Dell'Aquila et al., 2001; Tremoleda et al., 2003; Lewis et al., 2016). In fact, the PLCς has the greatest activity among the mammalian species that have been evaluated **(**Sato et al., 2013). While with utilization of ICSI procedures there is production of the same proportion of blastocysts with or without utilization of a PD, there is greater embryonic quality in terms of cell number and nuclear fragmentation rates in blastocysts produced when using PD ICSI procedures **(**Salgado et al., 2018).

Although the precise effect resulting from use of the PD remains to be determined, the use of this device seems to allow for a greater release of PLCς in the ooplasm, justifying utilization in several laboratories and potentially explaining the repeatability when ICSI procedures are conducted in horses **(**Galli et al., 2002; Choi et al., 2003; Foss et al., 2013). The localization and relatively greater activity of PLCς in horses may explain, at least in part, the repeatability of results when ICSI procedures are conducted using the **(**Galli et al., 2002; Choi et al., 2003; Bedford-Guaus et al., 2008, 2011; Foss et al., 2013, Sato et al., 2013). In cattle, the efficacy of ICSI is markably less than in horses **(**Katayose et al., 1999; Horiuchi et al., 2002; Devito et al., 2010); nonetheless, it is important to highlight that the PD was used for production of most of the ICSI calves **(**Horiuchi et al., 2002; Galli et al., 2003, Oikawa et al., 2005).

In pigs, the utilization of the PD was associated with greater alteration of the sperm plasma membrane, and the rate of formation pronuclei was markedly greater when the PD was used than with utilization of conventional ICSI **(**Katayama et al., 2006). The results from this study also indicated that microinjected matured oocytes had the capacity for full-term development when the PD was used to conduct ICSI without there being imposing of additional oocyte activation treatments.

Foals can be produced using ICSI procedures without using the PD **(**Rodríguez et al., 2019), and there have been no reports that this system is essential for ICSI to be an efficacious procedure. However, in most large-scale commercial horse ICSI enterprises use PD **(**Galli et al., 2002; Choi et al., 2002, 2004; Hinrichs et al., 2005; Foss et al., 2013).

2. ICSI Applications

The following analysis will focus on horses, a species for which ICSI is already feasible with excellent results and is being used increasingly by breeders in the horse industry. There will subsequently be a focus on use of ICSI in cattle. Although the current use is almost nonexistent in cattle due to the biological incompatibilities with use of ICSI, applications of ICSI have significant potential for valuable individuals. There will also be a brief description of the outcomes when there has been use of ICSI with pigs and small ruminants, for which the use of this technique has been limited.

2.1. Maximizing sperm use in animals with genetic value

Because the utilization of ICSI in horses requires a small number of spermatozoa, it has become a technique of interest to some owners who have a small number of frozen semen straws in inventory where the semen was collected from either older stallions or those that have died. There can, therefore, be proportioning of the semen contained in the straws into several aliquots in liquid nitrogen, and the reproportioned straw contents can subsequently be individually thawed, diluted at 1:200 and utilized for conducting ICSI procedures **(**Choi et al., 2006, 2011; Altermatt et al., 2009, Rader et al., 2016). Another interesting option that ICSI provides is the possibility of thawing the semen contents in a straw, using a portion of the contents, and then re-freezing the remaining contents in multiple straws containing semen doses that can be used for ICSI **(**Hamano et al., 1999; Rader et al., 2016; Canel et al., 2017).

With use of the ICSI procedure, there is also the possibility of using epididymal sperm. In humans, not only epididymal but also testicular sperm have been successfully used for ICSI as an alternative from ejaculated sperm in patients with cryptozoospermia **(**Silber et al., 1995; Kang et al., 2018). There has been birth of viable human neonates when using these procedures for conducting ICSI (Jin et al., 2020). In livestock species,

after an injury or death of a valuable male, recovery of epididymal spermatozoa could be the only option for ensuring gamete preservation. In horses, ICSI-derived embryos have been produced by using epididymal stallion semen that was previously cryopreserved **(**Vieira et al., 2013). Interestingly, results from a recent study indicated epididymal sperm of a zebra could also be utilized for activation of oocytes of domestic horses with there being embryo development to the blastocyst stage **(**Gambini et al., 2020).

With cattle, commercial bull semen processing enterprises have a priority to utilize semen from bull calves at the earliest possible age. It, therefore, is necessary to use semen from peripubertal bulls, which have a relatively lesser quantity and quality of sperm than reproductively mature bulls, thus both the semen quality and quantity problems when attempting to use semen from these bull calves can be solved with use of ICSI **(**Unnikrishnan et al., 2021). Furthermore, semen subjected to technological procedures that lead to a reduction in spermatozoa viability, such as freezing, re-freezing, or sorting by sex procedures, could be effectively utilized by conducting ICSI. For example, frozen thawed and refrozen bull sperm was used for ICSI without there being a detrimental effect on blastocyst production **(**Canel et al., 2017).

One of the sexes is preferred for certain animal production systems with examples being the preference for mares in polo and necessity for cows for dairy production. Sorting sperm by flow cytometry to enhance the quantity of X- or Y-bearing sperm in semen samples and utilization of these semen samples for artificial insemination (AI) has resulted in pregnancy rates of approximately 50% when there is use of fresh stallion semen samples that had been sex sorted for AI compared with only a 12% pregnancy rate when sex-sorted frozen-thawed stallion semen samples were used for AI **(**Squires et al., 2020). The ICSI procedure, therefore, is suitable for these types of samples where there is a very small number of spermatozoa with relatively lesser sperm motility as compared with semen samples for which sex sorting of sperm has not occurred **(**Iritani, 1991; Rath et al., 1999; Probst and Rath, 2003). Interestingly, with the use of ICSI there have been viable offspring produced even when only sperm heads were used to perform the technique **(**Hamano et al.,1999). With use of ICSI procedures, frozen, thawed, sex-sorted, and refrozen sperm have been used to produce foals (Carnevale et al., 2008).

In pigs, there is not as much interest in utilizing ICSI for maximizing the utilization of sperm because swine are one of the species with the largest semen volume **(**Bortolozzo et

al., 2015). Furthermore, boar sperm are extremely sensitive to cold temperatures, and, thus, less than 1% of all AI procedures are performed using frozen-thawed sperm (Thibier et al., 2002; Bailey et al., 2008;). When there is the use of frozen-thawed spermatozoa or frozen-thawed and sex-sorted spermatozoa to perform ICSI, several difficulties are incurred that are associated with slow rate of early embryonic development with there being few embryos developing to the blastocyst stage **(**Klocke et al.,1999). Only for boars with elite genetic value, therefore, for which only frozen samples are available or boars for which there is a small percentage of sperm with post-thaw motility, is ICSI a useful technique for producing offspring **(**Lai et al., 2001; Yong et al., 2006).

2.2. Overcoming quantitative and qualitative problems with sperm factors

As indicated previously in this review article, for humans, ICSI was developed to overcome pathologies that result in infertility as a result of sperm factors. The only requirement for ICSI is a spermatozoon with DNA integrity to fertilize an oocyte (Yanagimachi, 2005), but not acrosomal integrity and sperm motility. In humans, several pathologies associated with sperm can be overcome with use of ICSI **(**Palermo et al., 2017). One of these pathologies is globozoospermia, a structural abnormality associated with PLCζ deficiency and the absence of acrosomes, which can be overcome with normal birth of offspring resulting with the use of ICSI combined with a calcium ionophore treatment **(**Taylor et al., 2010; Fesahat et al., 2020). Furthermore, it has been ascertained that PLCζ is in lesser concentrations in spermatozoa of sub-fertile stallions (Gradil et al., 2006; Bedford-Guaus et al., 2012) and there is also a markedly lesser oocyte activation and embryo cleavage percentage when there is use of spermatozoa from theses sub-fertile stallions when conducting ICSI (Gonzalez-Castro 2019). The utilization of the ICSI procedure combined with artificial oocyte activation could be effective for production of offspring from these sub-fertile stallions with sub-optimal PLCζ concentrations.

There are 20% to 40% of bulls that have lesser fertility because of sperm abnormalities **(**Kastelic, 2013). In beef cattle, one of the main causes of abnormalities is the increase of testicular temperature to greater than optimal for sperm viability **(**2-6 °C). When there are these greater-than-optimal testicular temperatures, this lead to sperm hypoxia and morphology alterations, reducing the sperm viability and, therefore,

fertilization capacity. Common bull sperm compensable abnormalities (associated with sperm viability measures such as motility, acrosome integrity, and cell membrane integrity) can be overcome by increasing the semen dose (i.e., number of viable spermatozoa) used for AI. This approach in not feasible, however, when there are non-compensable spermatozoa defects (chromosomal abnormalities, protamine status, etc.). With these non-compensable spermatozoa abnormalities, the sperm have the capacities for fertilization and initiation of embryonic development, however, the spermatozoa do not have the capacity to support embryogenesis (reviewed by Kastelic, 2013). The utilization of ICSI when these circumstances prevail could be interesting, not necessarily for use of these individuals with these defects for reproduction purposes, but for basic research to elucidate which specific cellular processes that affect embryo development. Unlike in humans, where the use of ICSI for having offspring from a male parent with fertility problems is desirable, pursuing this objective in livestock species is not recommended because it may allow for the transmission of possible genetic problems to the offspring in the next generation (Steffen et al., 1997).

2.3. Increasing embryos from females with relatively greater genetic value

In horses, transvaginal ultrasonic-guided follicular aspiration (TVA) can be used for the collection of several immature oocytes and, in combination with ICSI, can be used to produce several *in vitro*-derived embryos per estrous cycle. Similar to embryo transfer **(**ET) of *in vivo*-derived embryos, the use of TVA-ICSI procedures combined can allow for the production of multiple foals from donor mares that are being used in competitive events without these mares not being available for competition during the gestational period, by transferring the embryos derived by using TVA-ICSI procedures into recipient mares **(**Squires et al., 2020). Several limitations associated with ET of *in vivo*-derived embryos were overcome with use of the combine TVA-ICSI procedures such as the collection of single embryos, restricted reproductive season and inefficiency resulting from the variable responses to treatment regimens for inducing multiple ovulations **(**Squires et al., 2007).

Conversely, the TVA-ICSI procedures can be performed throughout the year, and also during the period of reproductive transition into the breeding season. During this period the follicles are smaller, and the oocyte recovery rate is greater from follicles smaller than 10 mm because of the mechanical scraping of the follicle wall is more efficacious

when oocytes are being collected from smaller follicles **(**Cuervo-Arango et al., 2019). There also appears to be breed differences in follicle number **(**e.g., Arabian, Warmblood, and Quarter horse mares) **(**Galli et al., 2014) that presumably explains why average oocyte yield varies markedly from <5 to >12 oocytes for each TVA procedure **(**Jacobson et al., 2010; Galli et al., 2014; Cuervo-Arango et al., 2019). The interest in utilization of the TVA procedure increased markedly when Galli et al. (2016) reported recovering an average of 8.4 oocytes per TVA procedure that was performed.

Considering the previously described circumstances for use of ICSI procedures, breeders and trainers have a preference for the ICSI approach because the oocyte donor mare does not have to be inseminated and monitored to the extent as an embryo donor mare. The ICSI technology is used globally and an increasing number of veterinarians have developed the expertise for conducting TVA and delivery of immature oocytes to physical locations where ICSI procedures are conducted (Fig. 1). The commercial development of TVA-ICSI enterprises has resulted because of the demand for these procedures among sport horse breeders. Currently in Europe, the conducting of 60% of the TVA-ICSI procedures results in a blastocyst yield ranging from of 1.7 to 2 per procedural session and in 2019, there were about 5,000 embryos produced with use of TVA-ICSI procedures with an average pregnancy rate of 70% per transferred embryo at 17 days of the gestational period and a foaling rate in excess of 50%. **(**Lazzari et al., 2020). There was also an increase of the number of *in vitro*-derived embryos produced globally, according to the Data Retrieval Committee of the International Embryo Technology Society (2021). The data in these reports also indicated the number was larger for *in vitro*-derived frozen embryos than *in vivo*-derived frozen embryos (Fig. 2). The only limitation for further expansion is the cost of equipment and facilities and the shortage of technicians trained to perform TVA and ICSI procedures **(**Squires et al., 2020).

In cattle, the use of ICSI as a technique to obtain multiple embryos from females with relatively greater genetic value has been of less interest, and therefore, the ICSI procedure has not been extensively utilized **(**Oikawa et al., 2016), because IVF procedures have been efficacious for producing calves with enhanced genetic merit **(**Goto et al., 1990: Chung et al., 2000). There is also a lack of interest for developing

ICSI procedures for producing piglets because of the polytocous reproduction in this species.

2.4. Use of sub-fertile donors, prepubertal animals or females with sub-optimal oocyte quality

In horses, the TVA-ICSI procedures are effective in those mares with subfertility issues such as cervical, uterine or oviductal cancer, anatomical abnormalities, reproductive diseases, or mares that need to be euthanized or those that die suddenly **(**Hinrichs et al., 2005). One factor to consider is the age of the mares, with there being a markedly lesser oocyte recovery rate when mares are older **(**Stout, 2020).

With cattle, the use of prepubertal heifers as oocyte donors in breeding programs could decrease the generation interval and increase the genetic rate of gain when there is use of embryo transfer **(**Gordon, 1982; Georges and Massey, 1991). Cleavage and subsequent embryonic development rates are less after IVF using oocytes from prepubertal heifers as compared with oocytes from reproductively mature cows because in heifer calves there is an abnormal distribution of cortical granules resulting in a greater occurrence of polyspermy that is associated with deficient ooplasmic maturation **(**Levesque et al., 1996; Salamone et al., 2001). When there are these circumstances, ICSI could be utilized with there being subsequent induction of assisted oocyte activation as a possible alternative.

Oocytes from reproductively senescent cows have a disrupted distribution of cortical granules and as a consequence the process of fertilization is abnormal with a resulting lesser developmental competence of the oocytes when IVF procedures are conducted **(**Magata et al., 2019). In this study, with the use of ICSI there was not production of a larger percentage of blastocysts than with utilization of the IVF procedure, however, there was a larger proportion of oocytes from reproductively senescent cows developing into diploid blastocysts compared to when there was use of IVF for embryo production.

2.5. Facilitating the transport of oocytes and ICSI-derived embryos

In horses, ICSI allows for the use of oocytes that are maintained for a long period of time at room temperature. This facilitates the use of the combination of ICSI with TVA, which is usually performed at a physical location that is distant from the

micromanipulation laboratory. Immature oocytes can be "held" at room temperature for at least 24 h (Choi et al., 2006; Diaw et al., 2018) and transported while remaining viable in commercial embryo-holding media (Foss et al., 2013; Carnevale et al., 2016; Diaw et al., 2018) or in synthetic oviductal fluid (Galli et al., 2014). At room temperature, meiotic arrest is maintained overnight if oocytes are stored in an appropriate holding media. Temperatures between 4 and 10 °C, however, can have an effect on oocyte maturation, and “holding” at a temperature of 37 °C overnight can have an effect on embryo development, potentially because there is initiation of oocyte maturation when there are in maturation medium (Foss et al., 2013). Although there is an estimate of the optimal temperature range for maintenance of oocytes in the immature stage, there are still differences between laboratories, and an optimal temperature for oocyte maintenance in the immature state has not been firmly established. More research, therefore, is needed to establish, not only the temperature but also specific formulations, ensuring standardization of media and oocyte processing regimens (Metcalf et al., 2020) and the limits for maintaining and transporting immature oocytes. Until this is determined, it is probably prudent to transport oocytes at 20 to 22º C with there being “holding” for no longer than 36 h before transferring of oocytes into maturation medium (Stout, 2020).

As previously described in this review article, TVA-ICSI is frequently performed outside the physiological breeding season for horses and there is routine production of multiple embryos per TVA session. Cryopreservation of immature oocytes would allow for these oocytes to be thawed and fertilized at the convenience of the horse owner. Oocyte freezing, however, has been of limited efficacy, and vitrification of either horse or cattle oocytes results in a lesser fertilization, transfer, and ultimately pregnancy percentage relative to the number of vitrified oocytes on which ICSI was performed **(**Maclellan et al., 2002, 2010). After conducting ICSI procedures, therefore, *in vitro* embryo development must be conducted, and blastocysts can be cryopreserved until the time of embryo transfer. The ICSI-derived blastocysts **(**typically <300 µm diameter) can be cryopreserved using vitrification procedures **(**Choi et al., 2017) or slow freezing **(**Galli et al., 2007) with there being resulting pregnancy rates similar to those when there is transfer of fresh *in vitro*-derived embryos. There can even be transfer of large-expanded blastocysts **(**>300 µm) in which the blastocoel can artificially collapse that were vitrified using commercially available and economically affordable devices with there being very

acceptable pregnancy percentages when these large blastocysts are transferred into recipient females **(**Canesin et al., 2020). *In vitro*-derived embryos can be cryopreserved without a marked reduction in viability. In addition, embryos can be shipped and stored **(**preferable at temperatures from 15 to 18 °C for 12 to 24 h), until a recipient mare is available for transfer **(**Squires et al., 2016; Hinrichs, 2020).

In cattle, ICSI-derived embryos have a similar embryonic survival as IVF-derived embryos after freezing and thawing **(**Keskintepe and Brackett, 2000). Expanded ICSI-derived embryos **(**> 200 µm) are preferred for vitrification **(**Abdalla et al., 2010). In buffalo, when there was cryopreservation of mature oocytes and subsequent conducting of ICSI after oocyte thawing, there was embryo development to the blastocyst stage. There was a lesser percentage of oocytes on which ICSI was conducted that had a second PB formation, cleavage and that developed to the blastocyst stage compared to what occurred when ICSI was performed with freshly collected oocytes (Liang et al., 2011a, 2012). Additionally, after conducting ICSI there was also a lesser embryonic developmental competence of embryos derived from vitrified, as compared with freshly derived oocytes, of sheep and prepubertal goats (Hosseini et al., 2015; Menéndez-Blanco et al., 2020a). There, however, are no reports of cryopreserved ICSI-derived embryos from buffalo or small ruminants.

In pigs, results when there was a comparison of IVF, ICSI and "physiological ICSI" (PICSI, ICSI using hyaluronic acid) procedures resulted in percentage developments to the blastocyst stage from vitrified immature oocytes of 14%, 9% and 25%, respectively (Casillas et al., 2018). There are no reports of attempts to produce cryopreserved ICSI-derived embryos in pigs. Viable offspring were produced from cryopreserved IVF-derived embryos (Nagashima et al., 2007). Vitrification, therefore, is a potentially feasible technique for utilization of ICSI and PICSI procedures for pig embryo production.

2.6. Genome modification using ICSI

Intracytoplasmic sperm injection mediated-gene transfer **(**ICSI-MGT) has been used for exogenous transfer of genes combined with the sperm, before PN formation. This technique was used for production of transgenic embryos in livestock species such as cattle **(**Pereyra-Bonnet et al., 2008; Bevacqua et al., 2010; Canel et al., 2017; Sánchez-

Villalba et al., 2018; Sekhavati et al., 2018), ewes **(**Gou et al. 2002), goats **(**Shadanloo et al., 2010), horses **(**Zaniboni et al., 2013) and pigs **(**Lai et al., 2001; García-Vázquez et al., 2010). With this technique, there is utilization of sperm attached to exogenous DNA **(**transgen) and transport into the ooplasm when conducting the ICSI procedure, resulting in production of transgenic embryos. This technique is effective for ICSI in species such as pigs. There are more repeatable results for pigs, and several transgenic pigs have been produced using this technology **(**Yong et al. 2006; Kurome et al., 2006; Watanabe et al., 2012; Umeyama et al., 2012; Matsunari et al., 2014). Although advances were achieved with use of this technique, interest began to wane with emergence of the gene editing technique, CRISPR-Cas9 **(**Mojica et al., 2005; Barrangou et al., 2007; Jinek et al., 2012; Cong et al., 2013; Doudna and Charpentier, 2014; reviewed by Lander, 2016). Interestingly, it was reported by Ma et al. **(**2017) that when the CRISPR-Cas9 system was used when conducting ICSI in human metaphase II oocytes, there was a lesser occurrence of mosaicism in the resulting embryos. This finding indicates the targeting and efficiency of gene editing are associated with DNA synthesis and the phase of the cell cycle when the procedure is conducted **(**Hashimoto et al., 2016). Although ICSI in pigs is still a challenge to conduct, the excellent timing between zygote formation and the delivery of DNA using CRISPR warrants further study and is a promising approach for producing gene edited pig embryos.

3. The safety of ICSI

One aspect which should be considered regarding the safety of ICSI is the risk of transmitting genetic diseases and transmitting infectious diseases to the offspring. Similar to the concern in humans, one of the main concerns of ICSI in livestock species is that by conducting this procedure there is ignoring of the physiological barriers that allow for the elimination of defective sperm. This raises concerns about whether the use of this technique will result in production of offspring in which there is an increase in the incidence of disorders that are transmitted through the paternal genome. While ICSI is a technique to overcome male factor infertility, when fertilization failure has a genetic origin, with utilization of ICSI there may be transfer of the genetic basis for infertility to subsequent generations. In horses, there were no detrimental effects that were detected during the gestational period or in offspring when there were assessments by Valenzuela et al. (2017). It, however, is still necessary to determine in large scale studies whether

there are problems that occur as a result of using the ICSI procedure for producing offspring in livestock species.

Another concern is the transmission of infectious diseases when conducting ICSI procedures, which should be determined for each pathogen and species similar to what has occurred with use of many other reproductive technologies. Some aspects that can help in assessing the possible transmission is to determine if the pathogen is in the female reproductive tract during aspiration or in semen. Pathogens need to permeate the ZP to have effects at the oocyte membrane; and the pipette hole produced during ICSI could increase the risk of contamination when compared to when there is use of standard IVF procedures. For some pathogens the risks are significantly less, because the quantity of sperm and volume of insemination fluid is small. In addition, seminal plasma can be removed, and an antimicrobial treatment can be used when there is utilization of ICSI procedures **(**Bielanski, 2007).

4. ICSI in combination with other technologies

Several technologies that have been developed recently could be enhanced with the use of ICSI. Two of these are: novel technologies to preserve germplasm by lyophilization and the second involves production of offspring from germ cells produced *in vitro.*

Lyophilization has been widely used for gamete preservation, especially for sperm **(**Polge et al., 1949). The first reported ICSI with freeze-dried sperm was in the mouse **(**Wakayama and Yanagimachi., 1998) and there has been production of viable offspring with use of ICSI for deposition of freeze-dried sperm in oocytes **(**Wakayama and Yanagimachi., 1998; Kusakabe et al., 2001; Kaneko et al., 2003). This preceded use in large animals and, numerous authors have reported that there has been production of viable blastocysts with use of ICSI to deposit lyophilized sperm in pig **(**Kwon et al., 2004; Nakai et al., 2007), cattle **(**Lee et al., 2006), sheep (Olaciregui et al., 2017; Palazzese et al., 2018, 2020), and horse **(**Choi et al., 2011) oocytes. In a futuristic experiment by Wakayama et al. (2017), freeze-dried mouse sperm transported in a spaceship to the International Space Station. Sperm were stored for 9 months at –95 °C and exposed to space radiation, which is more than 100 times greater than that on earth. After these sperm were transported back to earth, the lyophilized sperm were deposited in mouse oocytes using ICSI procedures and there were viable offspring **(**similar to

control) produced by utilization of the freeze-dried sperm that had been transported and stored in the spaceship. Could freeze-dried sperm be the way to transport cattle genetics to other planets? If this occurs ICSI will likely be necessary for these sperm to be used for cattle production.

The ICSI procedure could be used to assist with use of new emerging technologies for enhancing reproduction of livestock, such as germ cell derivation from stem cells, that usually results in gametes with deficient characteristics, which do not have the capacity for fertilization. The production of mouse offspring from germ cells derived from stem cells **(**Hayashi et al., 2011, 2012) has resulted in the impetus for utilization of similar methodologies in farm animals **(**Goszczynski et al., 2019). In domestic animals, *in vitro* gametogenesis is still in early experimental stages of technique development; but theoretically, intergenerational intervals can be decreased by gamete derivation from stem cells and successive fertilization to accelerate genetic improvement. The production of stem cells to produce cattle embryos has occurred **(**Bogliotti et al., 2018), and in several studies there have been attempts to derive gametes from ovarian stem cells of cattle (de Souza et al., 2017), pluripotent stem cells of cattle and pigs **(**Malaver-Ortega et al., 2016; Wang et al., 2016, respectively) and embryonic stem cells of cattle **(**Shah et al., 2017). It, therefore, is possible to produce entire *in vitro* generations associated with the development of genomic technologies such as SNP arrays and next generation sequencing which could allow for accelerated genetic progress (Goszczynski et al., 2019).

5. ICSI challenges for livestock species

5.1. Horse oocyte cryopreservation

Currently, in horses, there is a focus on the vitrification of immature oocytes to preserve valuable mare genetics. In general, for domestic species, oocyte vitrification is not efficacious, potentially because of the large quantity of lipids within the oocytes. In horses, after warming the immature oocytes, the maturation and development percentages for embryos produced using ICSI remain small being 1% to 15% of sperm-injected oocytes that develop into blastocysts (Tharasanit et al., 2006; Canesin et al., 2017; Canesin et al., 2018). Pregnancy percentages are also small, with there having been only three live foals produced **(**Maclellan et al., 2002; Ortíz-Escribano et al., 2018).

In horses, as in other species, the intracellular oxidative state in sperm is affected by the vitrification and warming process, which results in increased reactive oxygen species (ROS) activity **(**Tatone et al., 2011; Zhao et al., 2015; Ren et al., 2015; Gutnisky et al., 2020). This sub-optimal oxidative state may partially explain the relatively lesser embryonic developmental percentages resulting when the vitrification and warming processes that spermatozoa undergo were used for ICSI (Canesin et al., 2017; Canesin et al., 2018**;** Ortiz-Escribano et al., 2018). Results from a recent study provide an impetus for optimism regarding the efficacy of utilization of ICSI procedures for livestock production (Clérico et al., 2021) after it was reported that when oocytes were supplemented with melatonin during *in vitro* maturation (IVM), there was a marked decrease in mitochondrial-specific ROS concentrations, both for vitrified and non-vitrified oocytes, with there being results similar to those for other species.

5.2. Offspring production when using ICSI in ruminants

The number of offspring produced using ICSI remains very few for ruminants **(**reviewed by García-Rosello et al., 2009; Lopez-Saucedo et al., 2012), with only 48 calves (Hamano et al.,1999; Wei and Fukui., 2002; Horiuchi et al; 2002; Galli et al., 2003; Oikawa et al.,2005;) 22 lambs (Catt et al., 1996; Gomez et al., 1998a, Porada et al., 2010) and one kid goat (Wang et al., 2003) having been reported. To the best of our knowledge, there have been no buffalo offspring produced using the ICSI procedure.

The ICSI procedure, therefore, has not yet been efficacious in ruminants (Gomez et al., 1998b; Chung et al., 2000; Pereyra -Bonnet et al., 2008; Shirazi et al., 2009; Devito et al., 2010, Arias et al., 2015) probably due to two primary problems: 1) decondensation of the sperm head (Rho et al., 1998; Chung et al., 2000; Malcuit et al., 2006b; Liang et al., 2011b; Chankitisakul et al., 2012; Arias et al., 2015,) and 2) infrequent oocyte activation **(**Malcuit et al., 2006a). There, therefore, have been two strategies evaluated with there being different approaches. One strategy focuses on the oocyte, with use of exogenous activation treatments to improve the percentage occurrences for oocyte activation and early embryo development. The other strategy is centered on developing sperm pretreatments to disrupt the sperm membrane and induce release of PLCζ to improve the formation of PN. The biological and methodological approaches with these possible strategies were described by Salamone et al. **(**2017), Unnikrishnan et al. **(**2021) and Valencia et al. (2021). In a recent report, there was a description of the sperm

"starvation and rescue method", in which sperm are maintained in the absence of nutrients until spermatozoa have the capacity for motility. Energy substrates are added back subsequently, “rescuing” sperm motility. Utilization of this method results in an increase in percentages of embryos developing to more advanced stages after fertilization (Navarrete et al., 2019).

Use of the PD device combined with utilization of ethanol-induced oocyte activation procedures after ICSI has resulted in production of the most calves (Horiuchi et al., 2002). There have been other approaches developed for utilization of ICSI in cattle that when there was use resulted in a greater percentage development of embryos to the blastocyst stage. One of these approached consists of using a large cysteamine concentration during IVM and sperm co-culture with cumulus oocyte complex prior to ICSI **(**Canel et al., 2018) and another approach consists of treating sperm with Hep-GSH **(**Canel et al., 2017). The efficacy of these approaches should be confirmed by the production of offspring after embryo transfer. In goats, the supplementation of maturation media with crocetin, a natural antioxidant constituent of saffron, has resulted in a reduction in concentrations of ROS in IVM-oocytes. These approaches, however, have not led to an improvement in the competence of prepubertal goat oocytes to develop to the blastocyst stage when there was use of ICSI for embryo production (Menéndez-Blanco et al., 2020b).

5.3. ICSI in pigs

The number of piglets produced as a result of use of ICSI in *in vitro*-matured oocytes is less than the number of piglets produced with use of ICSI in *in vivo* matured oocytes (Kolbe and Holtz 2000; Martin 2000; Lai et al., 2002; Nakai et al 2003; Probst and Rath 2003; Katayama et al. 2006). As with cattle, several boar sperm treatments have been evaluated in attempts to improve the efficacy of use of ICSI for piglet production (Katayama et al., 2002; Szczygiel and Ward, 2002; Yong et al., 2003; Lee and Yang, 2004; Tian et al., 2006; García-Roselló et al., 2006; García-Mengual et al., 2011; Casillas et al., 2018). Furthermore, there have been evaluations of the use of ICSI for piglet production by combining the use of this technique with assisted with electrical and chemical artificial stimulation of oocytes for activation (Kolbe and Holtz, 2000; Probs and Rath, 2003; García-Roselló et al., 2009). Although there are some reports that pretreatments and artificial activation are beneficial, Nakai et al. **(**2011) reported that

such pretreatments induce PLCz losses, resulting in a lesser sperm activation signal and consequently the capacity of the sperm to induce oocyte activation, Yong et al. (2006) reported that transgenic piglets can be produced without chemical or physical treatments of oocytes or sperm. In pigs, therefore, there is not a requirement for sperm pretreatment or artificial activation of the oocyte for piglet production.

6. Conclusions and future perspective

In humans, ICSI is the most widely utilized assisted reproductive technique. Recently, in horses, the use of this technology has been increasing rapidly and TVA-ICSI has proven to provide large benefits to the horse industry. The possibility of maintaining viable oocytes and embryos in a single media at room temperature, enables the bidirectional transportation between farm and laboratories where complex equipment is available for utilization, allowing many practitioners to use the technology. The utilization of the combined TVA-ICSI procedures has resulted in a consistent improvement in oocyte and embryo recovery rates resulting in a larger number of *in vitro*-derived, as compared with *in vivo*-derived embryos being cryopreserved, facilitating commercialization and propagation of the genetics of valuable animals.

In ruminants, although ICSI has various potential applications, such as the use of prepubertal animals to decrease the generation interval for genetic improvement, there are still biological limitations that have resulted in a lack of efficacy of this procedure for producing offspring. Future improvements, therefore, will be required for the efficacious use of ICSI in these species. In pigs, the use of the combination of the ICSI gene-editing techniques, such as CRISPR-Cas9, could be of primary importance in achieving genetic improvements for pork production. For all livestock species, ICSI could be used in combination with the new emerging technologies, such as lyophilized sperm and germ cell derivation from stem cells to enhance the production of offspring from genetically superior animals. The ICSI procedure, therefore, has emerged as a valuable technology, with rapid diffusion in the horse industry, which will likely lead to a greater use in other livestock species.

Conflict of interest

The authors report no declarations of interest.

Author contribution

Both authors contributed equally in the conceptualization, writing, and editing of this review.

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References

Altermatt, J.L., Suh, T.K., Stokes, J.E., Carnevale, E.M., 2009. Effects of age and equine follicle-stimulating hormone (eFSH) on collection and viability of equine oocytes assessed by morphology and developmental competency after intracytoplasmic sperm injection (ICSI). Reprod. Fertil. Dev. 21, 615–623.

Anzalone, D.A., Iuso, D., Czernik, M., Ptak, G., Loi, P., 2016. Plasma membrane and acrosome loss before ICSI is required for sheep embryonic development. J. Assist. Reprod. Genet. 33, 757–763.

Arias, M.E., Risopatrón, J., Sánchez, R., Felmer, R., 2015. Intracytoplasmic sperm injection affects embryo developmental potential and gene expression in cattle. Reprod. Biol. 15, 34–41.

Bailey, J.L., Lessard, C., Jacques, J., Brèque, C., Dobrinski, I., Zeng, W., Galantino-Homer, H.L., 2008. Cryopreservation of boar semen and its future importance to the industry. Theriogenology 70, 1251–1259.

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D. A., Horvath, P., 2007. CRISPR provides acquired resistance against viruses in prokaryotes. Science 315, 1709–1712.

Bavister, B.D., Yanagimachi, R.,1977. The Effects of Sperm Extracts and Energy Sources on the Motility and Acrosome Reaction of Hamster Spermatozoa in vitro. Biol. Reprod.16, 228–237.

Bedford-Guaus, S.J., Yoon, S.Y., Fissore, R.A., Choi, Y.H., Hinrichs, K., 2008. Microinjection of mouse phospholipase C zeta complementary RNA into mare oocytes induces long-lasting intracellular calcium oscillations and embryonic development. Reprod. Fertil. Dev. 20, 875–883.

Bedford-Guaus, S.J., McPartlin, L.A., Xie, J., Westmiller, S.L., Buffone, M.G., Roberson, M.S., 2011. Molecular cloning and characterization of phospholipase C

zeta in equine sperm and testis reveals species-specific differences in expression of catalytically active protein. Biol. Reprod. 85, 78–88.

Bedford-Guaus, S.J., McPartlin, L.A., Varner, D.D., 2012. Characterization of Equine Phospholipase C Zeta: A Review and Preliminary Results on Expression Defects in Subfertile Stallions. J. Equine Vet. Sci. 32, 445–450.

Bevacqua, R.J., Pereyra-Bonnet, F., Fernandez-Martin, R., Salamone, D.F., 2010. High rates of bovine blastocyst development after ICSI-mediated gene transfer assisted by chemical activation. Theriogenology 74, 922–931.

Bielanski, A., 2007. Disinfection procedures for controlling microorganisms in the semen and embryos of humans and farm animals. Theriogenology 68, 1–22.. .

Bogliotti, Y.S., Wu, J., Vilarino, M., Okamura, D., Soto, D.A., Zhong, C., Sakurai, M., Sampaio, R.V., Suzuki, K., Izpisua Belmonte, J.C., Ross, P.J., 2018. Efficient derivation of stable primed pluripotent embryonic stem cells from bovine blastocysts. Proc. Natl. Acad. Sci. U. S. A. 115, 2090–2095.

Bortolozzo, F.P., Menegat, M.B., Mellagi, A.P., Bernardi, M.L., Wentz, I., 2015. New artificial insemination technologies for Swine. Reprod. Domest. Anim. 50, 80–84.

Canel, N.G., Bevacqua, R.J., Hiriart, M.I., Rabelo, N.C., de Almeida Camargo, L.S., Romanato, M., de Calvo, L.P., Salamone, D.F., 2017. Sperm pretreatment with heparin and L-glutathione, sex-sorting, and double cryopreservation to improve intracytoplasmic sperm injection in bovine. Theriogenology 93, 62–70.

Canel, N. G., Suvá, M., Bevacqua, R. J., Arias, M. E., Felmer, R., Salamone, D. F., 2018. Improved embryo development using high cysteamine concentration during IVM and sperm co-culture with COCs previous to ICSI in bovine. Theriogenology 117, 26–33.

Canesin, H.S., Brom-de-Luna, J.G., Choi, Y.H., 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.

Canesin, H.S., Brom-de-Luna, J.G., Choi, Y.H., Pereira, A.M., Macedo, G.G., Hinrichs, K., 2018. Vitrification of germinal-vesicle stage equine oocytes: effect of cryoprotectant exposure time on in-vitro embryo production. Cryobiology 81, 185-191.

Canesin, H. S., Ortiz, I., Rocha Filho, A. N., Salgado, R. M., Brom-de-Luna, J. G., Hinrichs, K., 2020. Effect of warming method on embryo quality in a simplified equine embryo vitrification system. Theriogenology 151, 151–158.

Carnevale, E.M., 2004. Oocyte transfer and gamete intrafallopian transfer in the mare. Anim. Reprod. Sci. 82–83, 617–624.

Carnevale E. M., Graham J. K., Suh T. K., Stokes J. E., Squires E. L., 2008. Foals produced after ICSI using frozen, sex-sorted, refrozen sperm. Reprod. Fertil. Dev. 21, 228-228.

Carnevale, E.M., 2016. Advances in Collection, Transport and Maturation of Equine Oocytes for Assisted Reproductive Techniques. Vet. Clin. North Am. - Equine Pract. 32, 379–399.

Casillas, F., Betancourt, M., Cuello, C., Ducolomb, Y., López, A., Juárez-rojas, L., 2018. An efficiency comparison of different in vitro fertilization methods : IVF , ICSI , and PICSI for embryo development to the blastocyst stage from vitrified porcine immature oocytes 1–12.

Catt, S.L., Catt, J.W., Gomez, M.C., Maxwell, W.M.C., Evans, G., 1996. Birth of a male lamb derived from an in vitro matured oocyte fertilised by intracytoplasmic injection of a single presumptive male sperm. Vet. Rec. 139, 494–495.

Chankitisakul, V., Tharasanit, T., Phutikanit, N., Tasripoo, K., Nagai, T., Techakumphu, M., 2012. Lacking expression of paternally-expressed gene confirms the failure of syngamy after intracytoplasmic sperm injection in swamp buffalo (Bubalus bubalis). Theriogenology 77, 1415–1424.

Choi, Y.H., Love, C., Love, L., Varner, D., 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.

Choi, Y.H., Love, CC., Varner, D.D., Love LB., Hinrichs K., 2003. Effects of gas conditions, time of media change, and ratio of media to embryo on in vitro development of horse oocytes fertilized by intracytoplasmic sperm injection. Theriogenology 59, 19–29.

Choi, Y.H., Roasa, L.M., Love, C.C., Varner, D.D., Brinsko, S.P., Hinrichs, K. 2004. Blastocyst formation rates in vivo and in vitro of in vitro matured equine oocytes fertilized by intracytoplasmic sperm injection. Biol. Reprod. 70, 1231–1238.

Choi, Y.H., Love, L.B., Varner, D.D., 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.

Choi, Y.H., Varner, D.D., Love, C.C., Hartman, D.L., Hinrichs, K., 2011. Production of live foals via intracytoplasmic injection of lyophilized sperm and sperm extract in the horse. Reproduction 142, 529–538.

Choi, Y.H., Hinrichs, K., 2017. Vitrification of in vitro-produced and in vivo-recovered equine blastocysts in a clinical program. Theriogenology 87, 48-54.

Chung, J.T., Keefer, C.L., Downey, B.R., 2000. Activation of bovine oocytes following intracytoplasmic sperm injection (ICSI). Theriogenology 53, 73–84.

Clérico, G., Taminelli, G., Veronesi, J.C., Pololа, J., Pagura, N., Pinto, C., Sansinena, M., 2021. Mitochondrial function, blastocyst development and live foals born after ICSI of immature vitrified/warmed equine oocytes matured with or without melatonin. Theriogenology 160, 40–49.

Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P. D., Wu, X., Jiang, W., Marraffini, L. A., Zhang, F., 2013. Multiplex genome engineering using CRISPR/Cas systems. Science 339, 819–823.

Coy, P., Romar, R., 2002. In vitro production of pig embryos: A point of view. Reprod. Fertil. Dev. 14, 275–286.

Cuervo-Arango, J., Claes, A.N., Stout, T.A. 2019. A retrospective comparison of the efficiency of different assisted reproductive techniques in the horse, emphasizing the impact of maternal age. Theriogenology 132, 36-44.

Dell'Aquila, M.E., 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. Mol. Reprod. Dev. 60, 79–88.

Devito, L.G., Fernandes, C.B., Blanco, I.D.P., Tsuribe, P.M., Landim-Alvarenga, F.C., 2010. Use of a piezo drill for intracytoplasmic sperm injection into cattle oocytes activated with ionomycin associated with roscovitine. Reprod. Domest. Anim. 45, 654–658.

Diaw, M., Salgado, R.M., Canesin, H.S., Gridley, N., Hinrichs, K., 2018. Effect of different shipping temperatures (approximately 22 degrees C vs. approximately 7 degrees C) and holding media on blastocyst development after overnight holding of immature equine cumulus-oocyte complexes. Theriogenology 111, 62-68.

Doudna, J. A., Charpentier, E., 2014. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science 346, 6213.

Foss, R., Ortis, H., Hinrichs, K., 2013. Effect of potential oocyte transport protocols on blastocyst rates after intracytoplasmic sperm injection in the horse. Equine Vet. J. 45, 39–43.

Fesahat, F., Henkel, R., Agarwal, A., 2020. Globozoospermia syndrome: An update. Andrologia 52, 1–13.

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.

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.

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. Anim. Reprod. Sci. 98, 39–55

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.

Galli, C., Colleoni, S., Claes, A., Beitsma, M., Deelen, C., Necchi, D., et al., 2016. Overnight shipping of equine oocytes from remote locations to an ART laboratory enables access to the flexibility of ovum pick up-ICSI and embryo cryopreservation. J. Equine Vet. Sci. 41, 82.

Gambini, A., Rodriguez, M.B., Briski, O., Flores Bragulat, A., Demergasi, N., Losinno, L., Salamone, D.F., 2020. Ability of the zebra sperm to induce pig and horse oocyte activation and in vitro preimplantation embryo development after ICSI. J. Equine Vet. Sci. 89, 103052.

García-Mengual, E., García-Roselló, E., Alfonso, J., Salvador, I., Cebrian-Serrano, A., Silvestre, M. A., 2011. Viability of ICSI oocytes after caffeine treatment and sperm membrane removal with Triton X-100 in pigs. Theriogenology 76, 58–66.

García-Roselló, E., García-Mengual, E., Coy, P., Alfonso, J., Silvestre, M.A., 2009. Intracytoplasmic sperm injection in livestock species: An update. Reprod. Domest. Anim. 44, 143–151.

García-Roselló, E., Matás, C., Cánovas, S., Moreira, P.N., Gadea, J., Coy, P., 2006. Influence of sperm pretreatment on the efficiency of intracytoplasmic sperm injection in pigs. J. Androl. 27, 268–275.

Georges, M., Massey, J.M., 1991. Velogenetics, or the synergistic use of marker-assisted selection and germ line manipulation. Theriogenology 35, 151–159.

Gomez, M.C., Catt, J.W., Evans, G., Maxwell, W.M., 1998a. Cleavage, development and competence of sheep embryos fertilized by intracytoplasmic sperm injection and in vitro fertilization. Theriogenology 49, 43–54.

Gomez, M.C., Catt, J.W., Evans, G., Maxwell, W.M., 1998b. Sheep oocyte activation after intracytoplasmic sperm injection (ICSI). Reprod. Fertil. Dev. 10, 197–205.

Gonzalez-Castro, R.A., Amoroso-Sanches, F., Stokes, J.E., Graham, J.K., Carnevale, E.M., 2019. Localisation of phospholipase Cζ1 (PLCZ1) and postacrosomal WW-binding protein (WBP2 N-terminal like) on equine spermatozoa and flow cytometry quantification of PLCZ1 and association with cleavage in vitro. Reprod. Fertil. Dev. 31, 1778–1792.

Gordon, I., 1982. Synchronization of estrus and superovulation in cattle. Mammalian Egg Transfer, 63–80.

Goszczynski, D.E., Denicol, A.C., Ross, P.J., 2019. Gametes from stem cells: Status and applications in animal reproduction. Reprod. Domest. Anim. 54, 22–31.

Goto, K., Kinoshita, A., Takuma, Y., Ogawa, K., 1990. Fertilization of bovine oocytes by the injection of immobilized, killed spermatozoa. Vet. Rec. 139, 94–95.

Gou, K.M., An, X.R., Tian, J.H., Chen, Y.F., 2002. Sheep transgenic embryos produced by intracytoplasmic sperm injection. Shi Yan Sheng Wu Xue Bao 35, 103–108.

Gradil, C., Yoon, S., Brown, J., He, C., Visconti, P., Fissore, R., 2006. PLC zeta: a marker of fertility for stallions? Anim. Reprod. Sci. 94, 23–25.

Gutnisky, C., Morado, S., Gadze, T., Donato, A., Alvarez, G., Dalvit, G., et al., 2020. Morphological, biochemical and functional studies to evaluate bovine oocyte vitrification. Theriogenology 143, 18-26.

Hamano, K.I., Li, X., Qian, X.Q., Funauchi, K., Furudate, M., Minato, Y., 1999. Gender preselection in cattle with intracytoplasmically injected, flow cytometrically sorted sperm heads. Biol. Reprod. 60, 1194–1197.

Hashimoto, M., Yamashita, Y., Takemoto, T., 2016. Electroporation of Cas9 protein/sgRNA into early pronuclear zygotes generates non-mosaic mutants in the mouse. Dev. Biol. 418, 1–9.

Hayashi, K., Ohta, H., Kurimoto, K., Aramaki, S., Saitou, M., 2011. Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. Cell 146, 519–532.

Hayashi, K., Ogushi, S., Kurimoto, K., Shimamoto, S., Ohta, H., Saitou, M., 2012. Offspring from oocytes derived from in vitro primordial germ cell-like cells in mice. Science 338, 971–975.

Hinrichs, K., and Choi, Y.H., 2005. Update on equine ICSI and cloning. Theriogenology 64, 535–541.

Hinrichs, K., 2018. Assisted reproductive techniques in mares. Reprod. Domest. Anim. 53, 4–13.

Hinrichs, K., 2020. Advances in Holding and Cryopreservation of Equine Oocytes and Embryos. J. Equine Vet. Sci. 89, 102990.

Hiraoka, K., Kitamura, S., 2015. Clinical efficiency of Piezo-ICSI using micropipettes with a wall thickness of 0.625 μm. J. Assist. Reprod. Genet. 32, 1827–1833.

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.

Hosseini, S.M., Asgari, V., Ostadhosseini, S., Hajian, M., Ghanaei, H.R., Nasr-Esfahani, M.H., 2015. Developmental competence of ovine oocytes after vitrification: Differential effects of vitrification steps, embryo production methods, and parental origin of pronuclei. Theriogenology 83, 366–376.

Huang, T., Kimura, Y., Yanagimachi, R., 1996. The use of piezo micromanipulation for intracytoplasmic sperm injection of human oocytes. J. Assist. Reprod. Genet. 13, 320–328.

Hyakutake, T., Suzuki, H., Yamamoto, S., 2015. Effect of viscosity on motion

characteristics of bovine sperm. J. Aero Aqua Bio-mechanisms 4, 63–70.

International Embryo Tecnology Society, 2021 (IETS). Data Retrieval Committee Reports https://www.iets.org/Committees/Data-Retrieval-Committee.

Iritani, A., 1991. Micromanipulation of gametes for in vitro assisted fertilization. Mol. Reprod. Dev. 28, 199–207.

Jacobson, C. C., Choi, Y. H., Hayden, S. S., Hinrichs, K., 2010. Recovery of mare oocytes on a fixed biweekly schedule, and resulting blastocyst formation after intracytoplasmic sperm injection. Theriogenology 73, 1116–1126.

Jin, L., Li, Z., Gu, L., Huang, B., 2020. Neonatal outcome of children born after ICSI with epididymal or testicular sperm: A 10-year study in China. Sci. Rep. 10, 1–8.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., Charpentier, E., 2012. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337, 816–821.

Kaneko, T., Whittingham, D.G., Overstreet, J.W., Yanagimachi, R., 2003. Tolerance of the mouse sperm nuclei to freeze-drying depends on their disulfide status. Biol. Reprod. 69, 1859-1862.

Kang, Y.N., Hsiao, Y.W., Chen, C.Y., Wu, C.C., 2018. Testicular sperm is superior to ejaculated sperm for ICSI in cryptozoospermia: An update systematic review and meta-analysis. Sci. Rep. 8, 2–10.

Katayama, M., Miyano, T., Miyake, M., Kato, S., 2002. Progesterone treatment of boar spermatozoa improves male pronuclear formation after intracytoplasmic sperm injection into porcine oocytes. Zygote 10, 95–104.

Katayama, M., Zhong, Z., Lai, L., Sutovsky, P., Prather, R.S., Schatten, H., 2006. Mitochondrial distribution and microtubule organization in fertilized and cloned porcine embryos: implications for developmental potential. Dev. Biol. 299, 206–220.

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.

Keskintepe, L., Brackett, B.G., 2000. Cryopreservation of bovine blastocysts obtained by intracytoplasmic sperm injection. Theriogenology 53, 1041–1052.

Kimura, Y., Yanagimachi, R., 1995. Intracytoplasmic sperm injection in the mouse. Biol. Reprod. 52, 709–720.

Klocke, S., Rath, D., and Johson, L. A., 1999. Intracytoplasmic sperm injection (ICSI) of porcine oocytes with flowcytometrically sorted and unsorted semen. Reprod. Dom. Anim. 34, 35.

Kolbe, T., Holtz, W., 2000. Birth of a piglet derived from an oocyte fertilized by intracytoplasmic sperm injection (ICSI). Anim. Reprod. Sci 64. 97–101.

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 Res. 15, 229–240.

Kusakabe, H., Szczygiel, M.A., Whittingham, D.G., Yanagimachi, R., 2001. Maintenance of genetic integrity in frozen and freeze-dried mouse spermatozoa. Proc. Natl. Acad. Sci. U. S. A. 98, 13501–13506.

Kwon, I. K., Park, K. E., Niwa, K., 2004. Activation, pronuclear formation, and development in vitro of pig oocytes following intracytoplasmic injection of freeze-dried spermatozoa. Biol. Reprod .71, 1430–1436.

Lai, L., Sun, Q., Wu, G., Murphy, C. N., Kühholzer, B., Park, K. W., et al., 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, 339–346.

Lai, L., Kolber-Simonds, D., Park, K. W., Cheong, H. T., Greenstein, J. L., Im, G. S., et al., 2002. Production of alpha-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning. Science 295, 1089–1092.

Lander, E. S., 2016. The Heroes of CRISPR. Cell 164, 18–28.

Lazzari, G., Wrenzycki, C., Herrmann, D., Duchi, R., Kruip, T., Niemann, H., Galli, C., 2002. Cellular and molecular deviations in bovine in vitro-produced embryos are related to the large offspring syndrome. Biol. Reprod. 67, 767–775.

Lazzari, G., Colleoni, S., Crotti, G., Turini, P., Fiorini, G., Barandalla, M., Landriscina, L., Dolci, G., Benedetti, M., Duchi, R., Galli, C., 2020. Laboratory Production of Equine Embryos. J. Equine Vet. Sci. 89, 103097.

Lee, J.W.,Yang, X. 2004. Factors affecting fertilization of porcine oocytes following intracytoplasmic injection of sperm. Mol. Reprod. Dev. 68, 96–102.

Lee, K. B., Niwa, K., 2006. Fertilization and development in vitro of bovine oocytes following intracytoplasmic injection of heat-dried sperm heads. Biol. Reprod. 74, 146–152.

Levesque, J.T., Sirard, M.A., 1996. Resumption of meiosis is initiated by the accumulation of cyclin B in bovine oocytes. Biol. Reprod. 55, 1427–1436.

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.

Liang, Y.Y., Phermthai, T., Nagai, T., Somfai, T., Parnpai, R., 2011a. In vitro development of vitrified buffalo oocytes following parthenogenetic activation and intracytoplasmic sperm injection. Theriogenology 75, 1652–1660.

Liang, Y.Y., Ye, D.N., Laowtammathron, C., Phermthai, T., Nagai, T., Somfai, T.,

Parnpai, R., 2011b. Effects of Chemical Activation Treatment on Development of Swamp Buffalo (Bubalus bubalis) Oocytes Matured In Vitro and Fertilized by Intracytoplasmic Sperm Injection. Reprod. Domest. Anim. 46, 67–73.

Liang, Y.Y., Srirattana, K., Phermthai, T., Somfai, T., Nagai, T., Parnpai, R., 2012. Effects of vitrification cryoprotectant treatment and cooling method on the viability and development of buffalo oocytes after intracytoplasmic sperm injection. Cryobiology 65, 151–156.

Lopez-Saucedo, J., Paramio-Nieto, M.T., Fierro, R., Piña-Aguilar, R.E., 2012. Intracytoplasmic sperm injection (ICSI) in small ruminants. Anim. Reprod. Sci. 133, 129–138.

Ma, H., Marti-Gutierrez, N., Park, S. W., Wu, J., Lee, Y., Suzuki, K., et al., 2017. Correction of a pathogenic gene mutation in human embryos. Nature 548, 413–419.

Maclellan, L.J., Carnevale, E.M., Coutinho da Silva, M.A., Scoggin, C.F., Bruemmer, J.E., Squires, E.L., 2002. Pregnancies from vitrified equine oocytes collected from superstimulated and non-stimulated mares. Theriogenology 58, 911-919.

Magata, F., Tsuchiya, K., Okubo, H., Ideta, A., 2019. Application of intracytoplasmic sperm injection to the embryo production in aged cows. J. Vet. Med. Sci. 81, 84–90Malaver-Ortega, L. F., Sumer, H., Jain, K., Verma, P. J., 2016. Bone morphogenetic protein 4 and retinoic acid trigger bovine VASA homolog expression in differentiating bovine induced pluripotent stem cells. Mol. Reprod. Dev. 83, 149–161.

Malcuit, C., Kurokawa, M., Fissore, R.A., 2006a. Calcium oscillations and mammalian egg activation. J. Cell. Physiol. 206, 565–573.

Malcuit, C., Maserati, M., Takahashi, Y., Page, R., Fissore, R.A., 2006b. Intracytoplasmic sperm injection in the bovine induces abnormal [Ca2+]i responses and oocyte activation. Reprod. Fertil. Dev. 18, 39–51.

Martin, M.J., 2000. Development of in vivo-matured porcine oocytes following intracytoplasmic sperm injection. Biol. Reprod. 63, 109–112.

Matsunari, H., Kobayashi, T., Watanabe, M., Umeyama, K., Nakano, K., Kanai, T., 2014. Transgenic pigs with pancreas-specific expression of green fluorescent protein. J. Reprod. Dev. 60, 230–237.

McKinnon, A. O., Lacham-Kaplan, O., & Trounson, A. O., 2000. Pregnancies produced from fertile and infertile stallions by intracytoplasmic sperm injection (ICSI) of single frozen-thawed spermatozoa into in vivo matured mare oocytes. J. Reprod. Fertil. 56, 513–517

Menéndez-Blanco, I., Soto-Heras, S., Catalá, M.G., Piras, A.R., Izquierdo, D., Paramio, M.T., 2020a. Effect of vitrification of in vitro matured prepubertal goat oocytes on embryo development after parthenogenic activation and intracytoplasmic sperm injection. Cryobiology 93, 56–61

Menéndez-Blanco, I., Soto-Heras, S., Catalá, M.G., Roura, M., Izquierdo, D., Paramio, M.T., 2020b. Effect of crocetin added to IVM medium for prepubertal goat oocytes on blastocyst outcomes after IVF, intracytoplasmic sperm injection and parthenogenetic activation. Theriogenology 155, 70–76.

Metcalf, E.S., Masterson, K.R., Battaglia, D., Thompson, J.G., Foss, R., Beck, R., Cook, N.L., O'Leary, T., 2020. Conditions to optimise the developmental competence of immature equine oocytes. Reprod. Fertil. Dev. 32, 1012–1021.

Mojica, F. J., Díez-Villaseñor, C., García-Martínez, J., Soria, E., 2005. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. J. Mol. Evol. 60, 174–182.

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. Proc. Natl. Acad. Sci. U. S. A. 103, 17661–17666.

Nakai, M., Kashiwazaki, N., Takizawa, A., Hayashi, Y., Nakatsukasa, E., Fuchimoto, D., et al., 2003. Viable piglets generated from porcine oocytes matured in vitro and fertilized by intracytoplasmic sperm head injection. Biol. Reprod. 68, 1003–1008.

Nakai, M., Kashiwazaki, N., Takizawa, A., Maedomari, N., Ozawa, M., Noguchi, J., Kaneko, H., Shino, M., Kikuchi, K., 2007. Effects of chelating agents during freeze-drying of boar spermatozoa on DNA fragmentation and on developmental ability in vitro and in vivo after intracytoplasmic sperm head injection. Zygote 15, 15–24.

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.

Navarrete, F.A., Aguila, L., Martin-Hidalgo, D., Tourzani, D.A., Luque, G.M., Ardestani, G., et al., 2019. Transient Sperm Starvation Improves the Outcome of Assisted Reproductive Technologies. Front. Cell Dev. Biol. 7, 1–13.

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. Anim. Reprod. Sci. 86, 187–194.

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. J. Reprod. Dev. 62, 11–1.

Olaciregui, M., Luño, V., Domingo, P., González, N., Gil, L., 2017. In vitro developmental ability of ovine oocytes following intracytoplasmic injection with freeze-dried spermatozoa. Sci. Rep. 7, 1–8.

Ortíz-Escribano, N., Bogado Pascottini, O., Woelders, H., Vandenberghe, L., De Schauwer, C., Govaere, J., et al., 2018. An improved vitrification protocol for equine immature oocytes, resulting in a first live foal. Equine. Vet. J. 50, 391-397.

Palazzese, L., Gosálvez, J., Anzalone, D.A., Loi, P., Saragusty, J., 2018. Impairs Embryo Development 64.

Palazzese, L., Anzalone, D.A., Turri, F., Faieta, M., Donnadio, A., Pizzi, F., Pittia, P., Matsukawa, K., Loi, P., 2020. Whole genome integrity and enhanced developmental potential in ram freeze-dried spermatozoa at mild sub-zero temperature. Sci. Rep. 10, 1–10.

Palermo, G., Joris, H., Devroey, P., Van Steirteghem, A.C., 1992. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 340, 17–18.

Palermo, G.D., Cohen, J., Alikani, M., Adler, A., Rosenwaks, Z., 1995. Intracytoplasmic sperm injection: a novel treatment for all forms of male factor infertility. Fertil. Steril. 63, 9.

Palermo, G.D., Schlegel, P.N., Hariprashad, J.J., Ergün, B., Mielnik, A., Zaninovic, N., Veeck, L.L., Rosenwaks, Z., 1999. Fertilization and pregnancy outcome with intracytoplasmic sperm injection for azoospermic men. Hum. Reprod. 14, 741–748.

Palermo, G.D., O'Neill, C.L., Chow, S., Cheung, S., Parrella, A., Pereira, N., Rosenwaks, Z., 2017. Intracytoplasmic sperm injection: State of the art in humans. Reproduction 154, F93–F110.

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. Reprod. Fertil. Dev. 20, 741–749.

Polge, C., Smith, A.U., Parkes, A.S., 1949. Revival of spermatozoa after vitrification and dehydration at low temperatures. Nature 164, 666.

Porada, C.D., Sanada, C., Long, C.R., Wood, J.A., DesaI, J., Frederick, N., Millsap, L., Bormann, C., Menges, S.L., Hanna, C., Flores-Foxworth, G., Shin, T., Westhusin, M.E., Liu, W., Glimp, H., Zanjani, E.D., Lozier, J.N., Pliska, V., Stranzinger, G., Joerg, H., Kraemer, D.C., Almeida-Porada, G., 2010. Clinical and molecular characterization of a re-established line of sheep exhibiting hemophilia A. J. Thromb. Haemost. 8, 276–285

Porcu, E., Ciotti, P.M., Fabbri, R., Magrini, O., Seracchioli, R., Flamigni, C., 1997. Birth of a healthy female after intracytoplasmic sperm injection of cryopreserved human oocytes. Fertil. Steril. 68, 724–726.

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.

Rader, K., Choi, Y.H., Hinrichs, K., 2016. Intracytoplasmic Sperm Injection, Embryo Culture, and Transfer of In Vitro–Produced Blastocysts. Vet. Clin. North Am. - Equine Pract. 32, 401–413.

Rath, D., Long, C.R., Dobrinsky, J.R., Welch, G.R., Schreier, L.L., Johnson, L.A., 1999.

In vitro production of sexed embryos for gender preselection: high-speed sorting of X-chromosome bearing sperm to produce pigs after embryo transfer. J. Anim. Sci. 77, 3346–3352.

Ren, L., Fu, B., Ma, H., Liu, D., 2015. Effects of mechanical delipation in porcine oocytes on mitochondrial distribution, ROS activity and viability after vitrification. Cryo Letters 36, 30-36.

Rho, G.J., Wu, B., Kawarsky, S., Leibo, S.P., Betteridge, K.J., 1998. Activation regimens to prepare bovine oocytes for intracytoplasmic sperm injection. Mol. Reprod. Dev. 50, 485–492.

Rodríguez, M.B., Gambini, A., Clérico, G., Ynsaurralde-Rivolta, A.E., Briski, O., Largel, H., Sansinena, M., Salamone, D.F., 2019. Time of first polar body extrusion affects the developmental competence of equine oocytes after intracytoplasmic sperm injection. Reprod. Fertil. Dev. 31, 1805–1811.

Salamone, D. F., Damiani, P., Fissore, R. A., Robl, J. M., Duby, R. T., 2001. Biochemical and developmental evidence that ooplasmic maturation of prepubertal bovine oocytes is compromised. Biol. Reprod. 646, 1761–1768.

Salamone, D.F., Canel, N.G & Rodríguez, M. B., 2017. Intracytoplasmic sperm injection in domestic and wild mammals. Reproduction 154, 111–124.

Salgado, R.M., Brom-De-luna, J.G., Resende, H.L., Canesin, H.S., Hinrichs, K., 2018. Lower blastocyst quality after conventional vs. Piezo icsi in the horse reflects delayed sperm component remodeling and oocyte activation. J. Assist. Reprod. Genet. 35, 825–840.

Sánchez-Villalba, E., Elena, M., Loren, P., Fuentes, F., Pereyra-Bonnet, F., Salamone, D., Felmer, R., 2018. Improved expression of green fluorescent protein in cattle embryos produced by ICSI-mediated gene transfer with spermatozoa treated with streptolysin-O. Anim. Reprod. Sci. 196, 130–137.

Sato, K., Wakai, T., Seita, Y., Takizawa, A., Fissore, R.A., Ito, J., Kashiwazaki, N., 2013. Molecular characteristics of horse phospholipase C zeta (PLCζ). Anim. Sci. J. 84, 359–368.

Schalkoff, M.E., Oskowitz, S.P., Powers, R.D., 1989. Ultrastructural observations of human and mouse oocytes treated with cryopreservatives. Biol. Reprod. 40, 379–393.

Seita, Y., Ito, J., Kashiwazaki, N., 2009. Removal of acrosomal membrane from sperm head improves development of rat zygotes derived from intracytoplasmic sperm injection. J. Reprod. Dev. 55, 475–479.

Sekhavati, M.H., Hosseini, S.M., Tahmoorespur, M., Ghaedi, K., Jafarpour, F., Hajian, M., Dormiani, K., Nasr-Esfahani, M.H., 2018. PhiC31-based Site-Specific Transgenesis System for Production of Transgenic Bovine Embryos by Somatic Cell Nuclear Transfer and Intracytoplasmic Sperm Injection. Cell J. 20, 98–107.

Shah, S. M., Singla, S. K., Palta, P., Manik, R. S., Chauhan, M. S., 2017. Retinoic acid induces differentiation of buffalo (Bubalus bubalis) embryonic stem cells into germ cells. Gene 626, 358–366.

Shadanloo, F., Najafi, M.H., Hosseini, S.M., Hajian, M., Forouzanfar, M.,Ghaedi, K., Abedi, P., Ostadhosseini, S., Hosseini, L., Eskandari-Nasab,M.P., Esfahani, M.H., 2010. Sperm status and DNA dose play key roles in sperm/ICSI-mediated gene transfer in caprine. Mol. Reprod. Dev.77, 868–875.

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.

Silber, S.J., Devroey, P., Tournaye, H., Van Steirteghem, A.C., 1995. Fertilizing capacity of epididymal and testicular sperm using intracytoplasmic sperm injection (ICSI). Reprod. Fert. Develop. 7, 281–283.

Smits, K., Roels, K., Ververs, C., Van de Velde, M., Govaere, J., Van Soom, A., 2018. Fluorinert as an Alternative for Mercury in Piezo Drill Assisted ICSI in the Horse. J. Equine Vet. Sci. 66, 212.

Souza, G. B., Costa, J., da Cunha, E. V., Passos, J., Ribeiro, R. P., Saraiva, M., van den Hurk, R., Silva, J. 2017. Bovine ovarian stem cells differentiate into germ cells and oocyte-like structures after culture in vitro. Reprod. Domest. Anim. 52, 243–250.

Squires, E.L., McCue, P.M., 2007. Superovulation in mares. Anim. Reprod. Sci. 99, 1-8.

Squires, E.L., McCue, P.M., 2016. Cryopreservation of equine embryos. J. Equine Vet. Sci. 41, 7-12.

Squires, E., 2020. Current Reproductive Technologies Impacting Equine Embryo Production. J. Equine Vet. Sci. 89, 102981.

Steffen, D., 1997. Genetic causes of bull infertility. Vet. Clin. North Am. Food Anim. Pract. 13, 243–253.

Stout, T.A.E., 2006. Equine embryo transfer: Review of developing potential. Equine Vet. J. 38, 467–478.

Stout, T.A.E., 2020. Clinical Application of in Vitro Embryo Production in the Horse. J. Equine Vet. Sci. 89, 103011.

Szczygiel, M.A., Ward, W.S., 2002. Combination of dithiothreitol ans detergent treatment of spermatozoa causes paternal chromosomal damage. Biol. Reprod. 67, 1532-1537.

Takeuchi, S., Minoura, H., Shibahara, T., Shen, X., Futamura, N., Toyoda, N., 2001. Comparison of piezo-assisted micromanipulation with conventional micromanipulation for intracytoplasmic sperm injection into human oocytes. Gynecol. Obstet. Invest. 52, 158–162.

Tatone, C., Di Emidio, G., Barbaro, R., Vento, M., Ciriminna, R., Artini, P.G., 2011.

Effects of reproductive aging and postovulatory aging on the maintenance of biological competence after oocyte vitrification: insights from the mouse model. Theriogenology 76, 864-873.

Taylor, S.L., Yoon, S.Y., Morshedi, M.S., Lacey, D.R., Jellerette, T., Fissore, R.A., Oehninger, S., 2010. Complete globozoospermia associated with PLCζ deficiency treated with calcium ionophore and ICSI results in pregnancy. Reprod. Biomed. Online 20, 559–564.

Tharasanit, T., Colenbrander, B., Stout, T.A.E., 2006. Effect of maturation stage at cryopreservation on post-thaw cytoskeleton quality and fertilizability of equine oocytes. Mol. Reprod. Dev. 73, 627-637.

Tian, J.H., Wu, Z.H., Liu, L., Cai, Y., Zeng, S.M., Zhu, S.E., Liu, G.S., Li, Y., Wu, C.X., 2006. Effects of oocyte activation and sperm preparation on the development of porcine embryos derived from in vitro-matured oocytes and intracytoplasmic sperm injection. Theriogenology 66, 439-448.

Tremoleda, J.L., Van Haeften, T., Stout, T.A., Colenbrander, B., Bevers, M.M., 2003. Cytoskeleton and chromatin reorganization in horse oocytes following intracytoplasmic sperm injection: patterns associated with normal and defective fertilization. Biol. Reprod. 69, 186–194.

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. Mol. Reprod. Dev. 79, 218–228.

Unnikrishnan, V., Kastelic, J., Thundathil, J., 2021. Intracytoplasmic sperm injection in cattle. Genes (Basel). 12, 1–18.

Valencia, C., Pérez, F. A., Matus, C., Felmer, R., Arias, M. E., 2021. Activation of bovine oocytes by protein synthesis inhibitors: new findings on the role of MPF/MAPKs. Biol. Reprod. 104, 1126–1138.

Valenzuela, O. A., Couturier-Tarrade, A., Choi, Y. H., Aubrière, M. C., Ritthaler, J., Chavatte-Palmer, P., Hinrichs, K., 2018. Impact of equine assisted reproductive technologies (standard embryo transfer or intracytoplasmic sperm injection (ICSI) with in vitro culture and embryo transfer) on placenta and foal morphometry and placental gene expression. Reprod. Fertil. Dev 30, 371–379.

Vieira, L.A., Gadea, J., García-Vázquez, F.A., Avilés-López, K., Matás, C., 2013. Equine spermatozoa stored in the epididymis for up to 96h at 4°C can be successfully cryopreserved and maintain their fertilization capacity. Anim. Reprod. Sci. 136, 280–288.

Vincent, C., Pickering, S.J., Johnson, M.H., 1990. The hardening effect of dimethylsulphoxide on the mouse zona pellucida requires the presence of an oocyte and is associated with a reduction in the number of cortical granules present. J. Reprod. Fertil. 89, 253–259.

Thibier, M., Wagner, H.G., 2002. World statistics for artificial insemination in cattle.

Livest. Prod. Sci. 74, 203–212.

Wakayama, T., Yanagimachi, R., 1998. Development of normal mice from oocytes injected with freeze-dried spermatozoa. Nat. Biotechnol. 16, 639-641.

Wakayama, S., Kamada, Y., Yamanaka, K., Kohda, T., Suzuki, H., Shimazu, T., Tada, M.N., Osada, I., Nagamatsu, A., Kamimura, S., Nagatomo, H., Mizutani, E., Ishino, F., Yano, S., Wakayama, T., 2017. Healthy offspring from freeze-dried mouse spermatozoa held on the International Space Station for 9 months. Proc. Natl. Acad. Sci. U. S. A. 114, 5988–5993.

Wallach, E.E., Palermo, G.D., Cohen, J., Rosenwaks, Z., 1996. Intracytoplasmic sperm injection: a powerful tool to overcome fertilization failure. Fertil. Steril. 65, 899–908.

Wang, B., Baldassarre, H., Pierson, J., Cote, F., Rao, K.M., Karatzas, C.N., 2003. The in vitro and in vivo development of goat embryos produced by intracytoplasmic sperm injection using tail-cut spermatozoa. Zygote 11, 219–227.

Wang, H., Xiang, J., Zhang, W., Li, J., Wei, Q., Zhong, L., Ouyang, H., Han, J., 2016. Induction of Germ Cell-like Cells from Porcine Induced Pluripotent Stem Cells. Sci. Rep. 6, 1–13.

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 Res. 21, 605–618.

Wei, H., Fukui, Y., 2002. Births of calves derived from embryos produced by intracytoplasmic sperm injection without exogenous oocyte activation. Zygote 10, 149–153.

Yanagida, K., Hatayose, H., Yazawa, H., Kimura, Y., Konnai, K., Sato, A., 1998. The usefulness of a piezo-micromanipulator in intracytoplasmic sperm injection in humans. Hum. Reprod. 14, 448–453.

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. Hum. Reprod. 16, 148–152.

Yanagimachi, R., 2005. Intracytoplasmic injection of spermatozoa and spermatogenic cells: Its biology and applications in humans and animals. Reprod. Biomed. Online 10, 247–288.

Yong, H.Y., Pyo, B.S., Hong, J.Y., Kang, S.K., Lee, B.C., Lee, E.S., Hwang, W.S., 2003. A modified method for ICSI in the pig: Injection of head membrane-damaged sperm using a 3-4 μm diameter injection pipette. Hum. Reprod. 18, 2390–2396.

Yong, H.Y., Hao, Y., Lai, L., Li, R., Murphy, C.N., Rieke, A., Wax, D., Samuel, M., Prather, R.S., 2006. Production of a transgenic piglet by a sperm injection technique in which no chemical or physical treatments were used for oocytes or sperm. Mol.

Reprod. Dev. 73, 595–599.

Yoshida, N., Perry, A.C.F., 2007. Piezo-actuated mouse intracytoplasmic sperm injection (ICSI). Nat. Protoc. 2, 296–304.

Zambrano, F., Aguila, L., Arias, M.E., 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.

Zaniboni, A., Merlo, B., Zannoni, A., Bernardini, C., Lavitrano, M., Forni, M., Mari, G., Bacci, M.L., 2013. Expression of fluorescent reporter protein in equine embryos produced through intracytoplasmic sperm injection mediated gene transfer (ICSI-MGT). Anim. Reprod. Sci.137, 53–61.

Zhao, X., Min, J., Du, W., Hao, H., Liu, Y., Qin, T., et al., 2015. Melatonin enhances the in vitro maturation and developmental potential of bovine oocytes denuded of the cumulus oophorus. Zygote 23, 525-536.

Legends

Fig. 1. Schematic workflow of the sequence from TVA-ICSI to ET.

Fig. 2. Global *in vitro* and *in vivo,* fresh and frozen horse embryo transfers.

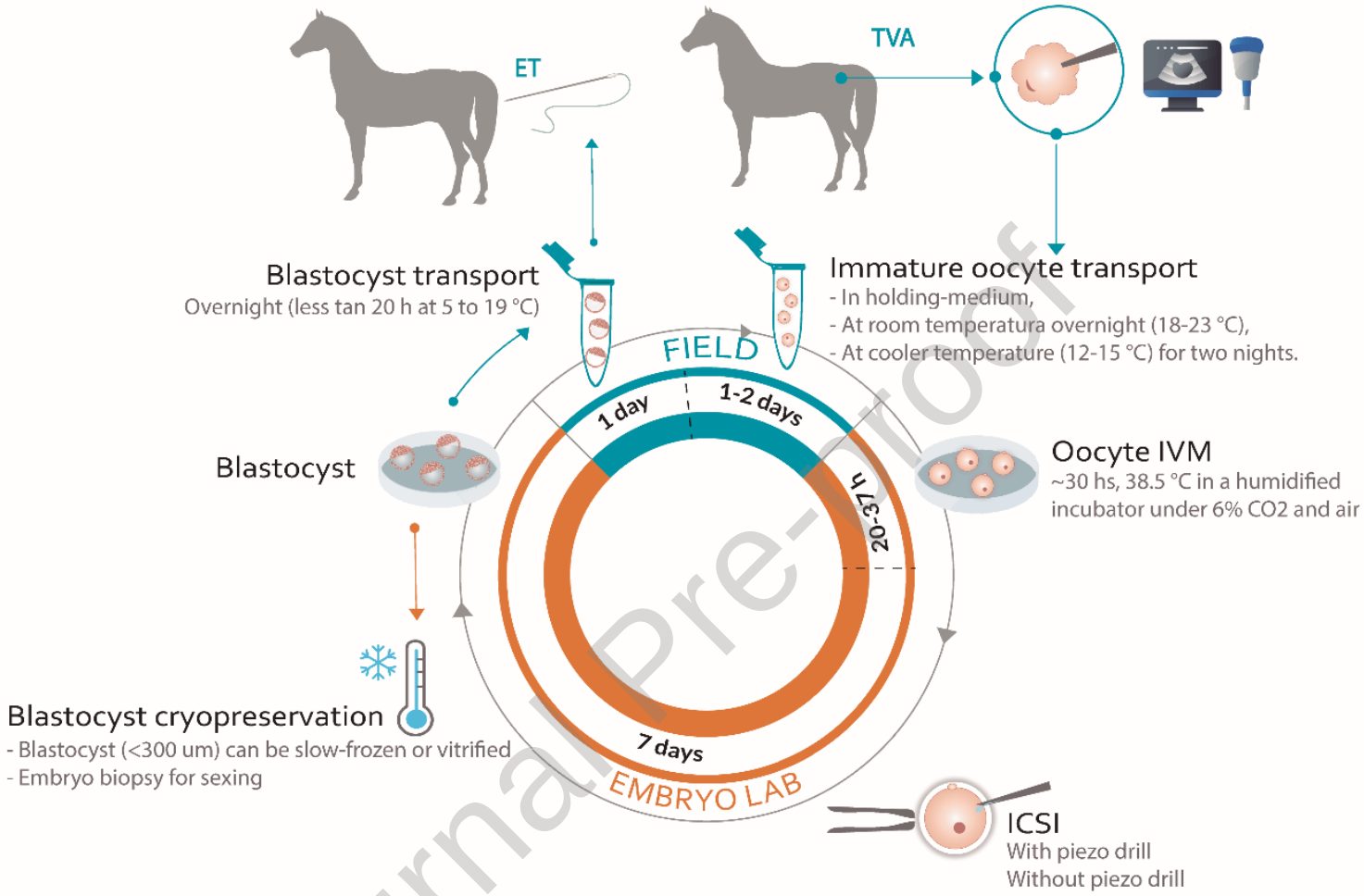
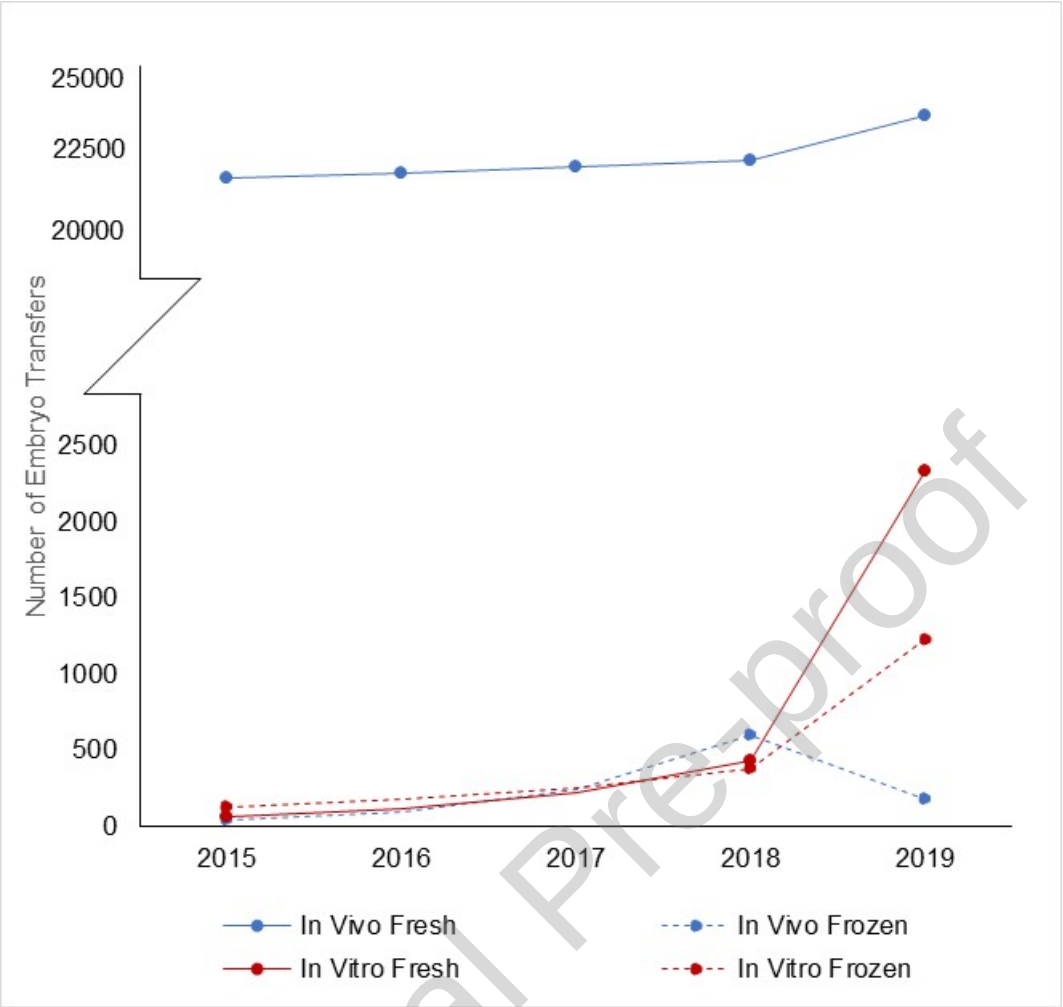


Figure 1

Note: specific procedures and recommended parameters can vary among laboratories.

Figure 2



Note: Report of data collected globally from 2015 to 2019 on horse embryo transfer (ET) from Data Retrieval Committee of The International Embryo Technology Society (IETS). Data for 2016 and 2017 are estimated based on an implicit consistent growth rate considering the data registered in 2015 and 2018.

Credit author statement

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Highlights

- ICSI has rapidly increased in use in horses, highly benefiting the equine industry.
- Currently, more equine *in vitro* embryos are frozen than *in vivo* embryos.

- In ruminants, future improvements are required for ICSI to be more widely used.
- In pigs, ICSI with gene-edition could be a valuable tool for genetic improvements.