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INVITED REVIEW

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Does in vitro application of pentoxifylline have beneficial effects in assisted male reproduction?

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Abstract

Application of nonspecific phosphodiesterases inhibitors, such as pentoxifylline (PTX), is a strategy utilised to aid sperm selection from immotile sperm samples prior to ICSI. No extensive studies have yet been performed to verify the safety of the clinical outcomes of ICSI after PTX administration. In this article, we summarise the data reported in the literature that assess the implication of in vitro usage of PTX on sperm parameters, as well as clinical outcomes during assisted male reproduction programme.

KEYWORDS

clinical outcome, embryo quality, male infertility, pentoxifylline, sperm parameters

1 | INTRODUCTION

Pentoxifylline (PTX) is a methylxanthine derivative that has received approval from the Food & Drug Administration (FDA) (Drugs@FDA, 2019). Its chemical structure is presented in Figure 1. Pentoxifylline is a vasoactive drug extensively used for treating vascular diseases (Donate-Correa et al., 2019). It has been used in treatment of male infertility, increasing sperm motility both in vivo (Azgomi et al., 2018) and in vitro (Table 1). Pentoxifylline not only improves sperm motility and plasmalemma integrity (Laokirkkiat, Kunathikom, Choavaratana, Petyim, & Prechapanich, 2007; Ponce et al., 1999), but also increases sperm cervical mucus penetration (Nassar et al., 1999b). Pentoxifylline belongs to the family of phosphodiesterase inhibitors (PDE) that leads to an increase of the cyclic adenosine monophosphate (c-AMP) levels. This leads to an increased number of acrosome reactions as well as sperm movement characteristics (Esteves, Spaine, & Cedenho, 2007; Nassar et al., 1998; Salian et al., 2019). PTX also shows antioxidant characteristics (Chehab, Madala, & Trussell, 2015) and, via prohibiting xanthine oxidase, leads to a reduction of the levels of intracellular reactive oxygen species (ROS) produced by defective spermatozoa, also diminishing lipid peroxidation (McKinney, Lewis, & Thompson, 1996). Though ROS are crucial for typical sperm function, at high rates, they adversely affect sperm membrane and genome integrity. Furthermore, enhanced lipid peroxidation reduces membrane fluidity, leading to diminished sperm motility and deficiency of sperm essential functions, such as the acrosome reaction (Alahmar, 2019). Both the antioxidative activity and radical scavenging characteristics of PTX have been shown to improve the likelihood of acrosome reactions (Esteves et al., 2007; Salian et al., 2019; Williams & Ford, 2005) and hyperactivation of spermatozoa (Kay, Coutts, & Robertson, 1993; Nassar et al., 1998; Pang, Chan, & Lu, 1993). Treatment of sperm samples with PTX is done in two different ways: 1, with addition of certain volume (depending on the final concentration) of PTX stock to the resuspended sperm pellet (Amer, Metawae, Hosny, & Raef, 2013; Salian et al., 2019); 2, addition of PTX solution that has been prepared at final concentration to the microdroplets of an ICSI Petri dish (Navas et al., 2017) (Figure 2).

Despite the above summarised PTX properties, there are, however, a limited amount of published data about the influence of laboratory application of PTX on intracytoplasmic sperm injection (ICSI) clinical outcomes. The purpose of this review is to collect, group and summarise the published studies that assessed the influence of

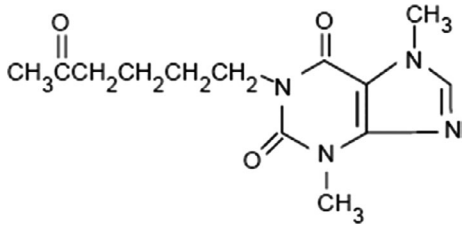

FIGURE 1 The structural formula of PTX

laboratory utilisation of PTX on sperm parameters and clinical outcomes in assisted male reproduction programmes.

2 | METHODOLOGY

We searched MEDLINE–PubMed (http://www.ncbi.nlm.nih.gov/PubMed), Google Scholar (https://scholar.google.com/), Scopus (https://www.scopus.com) and ISI web of science (http://apps.lib.

TABLE 1 Impact of in vitro PTX usage in improving sperm motility in ART programme

PTX dosage and incubation time	Conclusion	Authors
1 mg/ml (3.6 mmol/L) PTX and 45-min incubation at 37°C	After PTX treatment in oligospermic samples, sperm concentration and motility significantly improved, but no significant differences were noted for the normospermic samples	(Yovich, Edirisinghe, Cummins, & Yovich, 1988).
1 mg/ml PTX and 10-min incubation at 37°C	PTX raised the curvilinear, path, straight-line velocities, lateral head displacement, beat cross frequency and sperm hyperactivation both in normozoospermic and in asthenozoospermic specimens	(Tesarik, Thebault, & Testart, 1992)
2 mg/ml PTX and 30-min incubation at 37°C	PTX-enhanced acrosome reaction and sperm motility in cases with fertilisation failure or very low fertilisation rate (<10%)	(Tasdemir, Tasdemir, Kodama, & Tanaka, 1993)
4 mmol/L PTX and 0 and 4-hr incubation	PTX, firstly stimulated sperm motion in teratozoospermic samples. However, after 4-hr incubation, insignificant effect was detected. On the other hand, no improvement in zona pellucida binding potential was observed.	(Kaskar, Franken, van der Horst, & Kruger, 1994)
1 mg/ml PTX and 60-min incubation at 37°C	PTX improved spermatozoa motility in normo- and asthenozoospermic samples.	(McKinney, Lewis, & Thompson, 1994)
3.6 mmol/L PTX then 15-, 30-, 60-, 120- and 180-min incubation at 37°C	In asthenozoospermic patients, PTX improved both the numbers of motile spermatozoa and quality of movement over the first 60 min. But, after 120 min, there was a significant decrease in progressively motile spermatozoa in both control and PTX-treated groups.	(Lewis, McKinney, & Thompson, 1994)
3.6 mmol/L PTX and 30-min incubation at 37°C	When spermatozoa were damaged by the presence of antisperm antibodies, use of PTX improved sperm motility and induced acrosome reaction.	(Verheyen et al., 1994)
0.7, 1.5 and 3.6 mmol/L then 45-min incubation at 37°C	Motile sperm concentration after treatment of samples with 1.5 and 3.6 mmol/L of PTX improved, but the per cent of abnormal forms was unaffected.	(Mladenovic, Micic, Pearson, Genbacev, & Papic, 1994)
1 mg/ml PTX and 40-min incubation at 37°C	PTX improved sperm progressive motility in asthenospermic patients.	(Dimitriadou et al., 1995)
1 mmol/L PTX and 30-min incubation at 37°C	PTX decreased ROS formation and preserved sperm motion in vitro.	(Okada et al., 1997)
3.5 mmol/L PTX and 45-min incubation at 37°C	PTX had effective role on sperm motility in fresh ejaculates, cryopreserved ejaculates, epididymal and testicular spermatozoa from asthenozoospermic patients	(Khalili, Vahidi, & Fallah-Zadeh, 2001)
1 mg/ml PTX for 10, 30 then 60-min incubation at 37°C	The combined use of PTX and glycyrrhiza glabra acted as sperm motility stimulants in conditions of male infertility.	(Al-Dujaily & Malik, 2013)
1.76 mmol/L PTX and 15-min incubation	PTX and platelet-activating factor caused an increase of sperm movement.	(Archer & Roudebush, 2013)
5 mmol/L PTX and 1-hr. incubation	PTX increased spermatozoa motion by means of increasing CK activity of sperm cell in normal semen samples.	(Banihani & Abu-Alhayjaa, 2016)
5 and 10 mmol/L PTX then, 1-hr. incubation at 25°C in darkness	Adding 5 mmol/L PTX to sperm samples significantly enhanced the progressive motility, but adding 10 mmol/L PTX had negative effects.	(Banihani et al., 2018)
1 mmol/L PTX and 60-min incubation at 37°C, motility was assessed at various intervals (1, 4, 24, 48, and 72 hr).	In normozoospermia, 1 hr. after incubation, significant enhancement in total motility observed in PTX samples, but it decreased gradually with incubation time. In asthenozoospermia, at 1-hr. interval, the percentage of total motile spermatozoa did not rise significantly, but progressive motility was significantly higher than control.	(Salian et al., 2019)

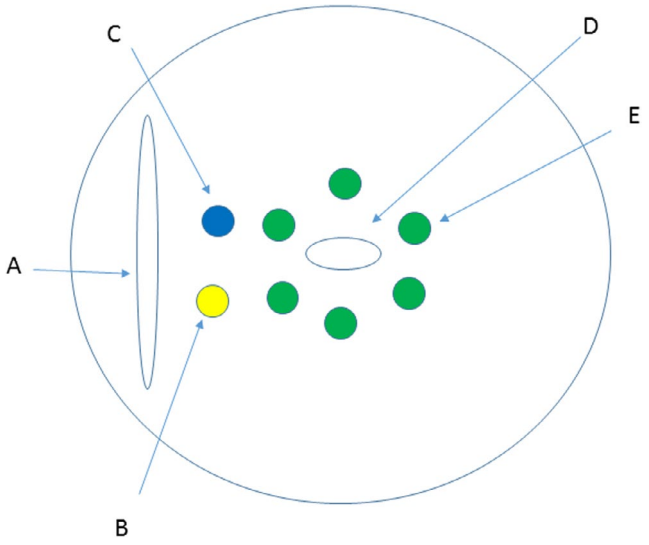

FIGURE 2 Sperm preparation and injection dish. On one side of the dish, elongated drop of sperm sample is deposited. In this drop, the sperm selection is performed with an injection needle. The number of elongated drops depends on the quality of the sample. (a) Sperm aliquot droplet in addition to prepared PTX; after incubation time, morphologically normal and motile sperm cells are picked using the microinjection pipette and placed in sperm washing droplet. (b) Sperm washing droplet. (c) Host-selected sperm droplet; selected spermatozoa are stored here until the ICSI procedure. (d) PVP droplet; immediately before the ICSI procedure, the sperm cells in C droplet are transferred into D droplet. (e) Oocyte injection droplets

wosg.ir/WOS) databases, from the earliest available online indexing year until February 2020 using the following Medical Subject Headings (MeSH) and non-MeSH keywords: 'Pentoxifylline', 'male infertility', 'sperm motility', 'sperm morphology', 'sperm vitality', 'sperm DNA integrity', 'embryo quality', and 'clinical outcome'. The searches were limited to the original articles published in English and examined the in vitro application of PTX in the treatment of male infertility. We excluded the in vivo studies as well as animal studies.

3 | PTX AND SPERM MOTILITY

Investigations related to correlation between sperm parameters with ICSI results disclosed that when only one immotile spermatozoon (seemingly dead) was injected into the oocyte, it negatively influenced the results of ICSI (Liu et al., 1995). Application of PTX after thawing has been shown to improve the post-thaw motility of cryopreserved sperm samples (Table 2). The mechanism for enhancing sperm motility has not yet been completely established, although PTX seems to enhance the activity of seminal creatine kinase (CK) (Banihani & Abu-Alhayjaa, 2016) and the amount of nitric oxide (NO) formed by sperm cells (Banihani, Abu-Alhayjaa, Amarin, & Alzoubi, 2018). Creatine kinase accelerates the regeneration of ATP from the chemical shuttle between creatine and creatine phosphate (Banihani & Abu-Alhayjaa, 2016). In vitro stimulation of sperm motility relies on the augmented intracellular level of cAMP (Buffone, Wertheimer, Visconti, & Krapf, 2014). Pentoxifylline is able to increase the intracellular levels of cAMP and NO could stimulate the guanylate cyclase (cGMP) pathway, which has an important function in the male's reproductive tract as well as sperm movement, chemotaxis and sperm-zona pellucida binding capacity. Nitric oxide may therefore moderate the stimulatory effect of PTX on sperm motion (Banihani et al., 2018).

4 | PTX AND SPERM VITALITY

Microinjection of nonmotile spermatozoa during ICSI negatively influences fertilisation, pregnancy and implantation rates (Nagy et al., 1998; Nordhoff, 2015; Verheyen et al., 2004). One vitality assay relies upon this fact that dead sperm cells absorb the supravitally red stain of Eosin Y; while, alive spermatozoa will be unstained. Use of eosin Y staining, however, leads to the loss of the spermatozoa in clinical setting negating its use in ICSI cycles. Nontoxic vitality tests are required when immotile spermatozoa are the only existing source for ICSI. Use of PTX involves addition to the sperm samples pre-ICSI, to stimulate the motility via the prohibition of 3′,5′-nucleotidase phosphodiesterase therefore increasing the concentration of intracellular cyclic nucleotides (Nassar et al., 1999a). By using PTX for sperm selection in cases with previous total fertilisation failure following ICSI (Goksan Pabuccu, Sinem Caglar, Dogus Demirkiran, & Pabuccu, 2016) or complete immotile spermatozoa from surgical sperm retrieval, the mean time needed to complete the ICSI procedures was reduced, due to enhanced and faster detection of viable, motile spermatozoa (Kovacic, Vlaisavljevic, & Reljic, 2006). Therefore, PTX enhances sperm motility, differentiating viable sperm cells, and improves the chance of fertilisation (Khalili, Mir-Rokni, & Kalantar, 1999). Table 3 summaries the studies about effectiveness of PTX usage in selecting viable spermatozoa in ART programmes.

5 | PTX AND SPERM MORPHOLOGY

Sperm motility is affected by sperm morphology. The morphology of spermatozoa mostly refers to the shape of the head, the length of the flagellum segments, including the connecting piece (also termed the neck), mid-piece, principal piece and end piece, and the size of the accessory structures, including axonemes, outer dense fibres (ODFs), mitochondrial sheath (MS) and fibrous sheath (FS) (Gu, Zhao, Wang, & Sun, 2019). The nucleus occupies a main part of the sperm head and contains condensed chromatin (Ward, 2018). Morphological defects of the spermatozoa termed teratozoospermia, which is distinguished by less than 4% of normal shape spermatozoa in the ejaculate according to the World Health Organization (WHO) reference value (Touré et al., 2020). While head morphology is essential for spermatozoa–oocyte interaction; normal sperm tail morphology seems to be the most essential factor in ensuring sperm motility (Lehti & Sironen, 2017).

TABLE 2 Impact of in vitro PTX usage in improving motility of devitrified sperm cells in ART programme

PTX dosage and incubation time	Conclusion	Authors
3.5 mmol/L PTX, progressive motility was detected at initially, 15, 60, 120 min and 24 hr. post-thaw	There was significant improvement of mean motility at 15, 60 and 120 min post-thaw. But no significant post-thaw viability was reported at 24 hr.	(Buch, Philips, & Kolon, 1994)
1, 3 and 10 mmol/L PTX and incubated at 37°C for 10 min	Cryoprotectant supplemented with 1 mmol/l PTX improved post-thaw progressive motility and total motility without reducing the percentage of spermatozoa with normal acrosomal regions.	(Brennan & Holden, 1995)
3.6 mmol/L PTX and incubation at 15 min, 1, 2 and 24 hr. post-thaw	Addition of PTX to semen of spinal cord injured men seemed to be suitable at 1-hr. post-exposure in the cryopreserved samples and in vitro effectiveness of PTX decreased prior to 24 hr. post-thaw.	(Kolon, Philips, & Buch, 1995)
3 mmol/L PTX and 30-min incubation at 37°C	Addition of PTX to fresh semen prior to cryopreservation significantly reduced progressive and total motility compared with controls, while addition of PTX to cryopreserved semen after thawing significantly enhanced progressive and total motility compared with controls.	(Stanic, Sonicki, & Suchanek, 2002)
5 mmol/L PTX incubated for 30 min at 37°C	Exposure of poor quality spermatozoa with PTX before cryopreservation did not increase post-thaw motility and viability throughout the freeze–thaw procedure.	(Esteves et al., 2007)
3.6 mmol/L PTX and 30-min incubation at 37°C	Exposure of devitrified sperm cells with PTX-enhanced motility in asthenozoospermia cases.	(Nabi, Khalili, Fesahat, et al., 2017)
3.6 mmol/L PTX (1 ml/1 mg) and 30-min incubation at 37°C	After thawing, PTX significantly enhanced the occurrence of the progressive spermatozoa in comparison with L-carnitine in infertile men.	(Aliabadi, Jahanshahi, Talaei-Khozani, & Banaei, 2018)

Teratozoospermia is concomitant with an extensive range of sperm tail morphological deficiencies, such as abnormal mitochondrial sheath, abnormal head–tail or mid-piece–principal piece junction, abnormal tail bending or coiling, irregular tail calibre or abnormal residual cytoplasm. The most severe form of morphological deficiencies is entire or near lack of the tail, a phenotype termed 'short tails' or 'stump tails' (Touré et al., 2020). If the reason of sperm immotility is not a structural abnormality, PTX can be applied, as reported above, for identification of alive testicular spermatozoa to increase fertilisation and pregnancy rates (Tasdemir, Tasdemir, & Tavukcuoglu, 1998). On the contrary, sperms that are lacking mid-piece will not demonstrate any type of motility even after PTX exposure (Khalili et al., 1999). It has been speculated that a limited number of spermatozoa with normal forms respond to PTX, even though nearly all PTX-affected spermatozoa demonstrated abnormal ultrastructural morphology (Hattori et al., 2011). Studies by means of electron microscopy displayed that freezing has damaging consequences on sperm cells, mainly on plasma membrane, acrosomes and tail (Ozkavukcu, Erdemli, Isik, Oztuna, & Karahuseyinoglu, 2008). In 2017, Nabi et al. described for the first time, the relationship between post-thaw sperm ultrastructure and administration of the PTX in asthenozoospermic samples. Their results revealed that PTX inverts the negative consequences of cryopreservation on sperm motion. In addition, PTX did not induce specific ultrastructural alterations in human vitrified spermatozoa (Nabi, Khalili, Talebi, et al., 2017) (Table 4).

6 | PTX AND SPERM DNA INTEGRITY

Both morphology and motility are correlated with sperm DNA impairment (Meseguer et al., 2012), with diminished sperm motility concomitant with an elevation of sperm genome impairment (Elbashir et al., 2018). Specific nuclear deficiencies of spermatozoa defined as 'vacuole-like structures' related to DNA integrity, failures of chromatin condensation, aneuploidy and differences in the DNA methylation levels (Cassuto et al., 2016). Assessment of sperm motion could be significant in predicting modifications in DNA (Unsal et al., 2016). On the other hand, sperm DNA fragmentation assays offer accurate evidence about genome integrity that may assist the medical staff to recognise the origin of infertility (Elbashir et al., 2018). Even though, the effectiveness of PTX on DNA integrity is doubtful, based on some studies (Ghasemi et al., 2014; Meseguer et al., 2009; Unsal et al., 2016), other investigations have indicated that PTX may be capable of rectifying sperm motility, deprived of any hostile impacts on sperm chromatin/DNA integrity (Table 5). Since sperm cells have compressed chromatin structure, some authors used additional time to distinguish DNA fragmentation (Asokan et al., 2015; Salian et al., 2019).

TABLE 3 Effectiveness of PTX usage in selecting viable spermatozoa in ART programme

PTX dosage and incubation time	Conclusion	Authors
3.6 mmol/L PTX then 30-, 60- and 90-min incubation at 37°C	PTX augmented the sperm motility significantly in TESE samples: 51.8 ± 10.2, 64.4 ± 9.4 and 70.8 ± 8.9% at 30, 60 and 90 min, respectively. Therefore, it can distinguish viable testicular spermatozoa during ICSI to improve pregnancy rates.	(Tasdemir et al., 1998)
3 mmol/L PTX for 30-min incubation at 37°C	After in vitro use of PTX, the motility of immotile testicular spermatozoa was improved or initiated.	(Angelopoulos et al., 1999)
5 mmol/L PTX for 10-min incubation	In cases with total lack of sperm motility, in situ use of PTX improved sperm assessment.	(de Mendoza et al., 2000)
1 mg/ml (3.6 mmol/L) and 30-min incubation (37°C)	In cases of 100% asthenozoospermia, PTX had the ability to differentiate live spermatozoa.	(Khalili et al., 1999)
7.2 mmol/L PTX and 15- to 20-min incubation	Application of PTX to frozen–thawed immotile testicular spermatozoa in cases of nonobstructive azoospermia (NOA) was necessary to stimulate motility.	(Giorgetti et al., 2005)
1.76 mmol/L PTX and 20-min incubation at 37°C	In cases of immotile testicular spermatozoa, the use of PTX caused easier identification of vital spermatozoa, improved fertilisation rates and embryos development.	(Kovacic et al., 2006)
1.5 mmol/L PTX and 10-min incubation	PTX permits the selection of viable testicular frozen–thawed spermatozoa from patients with NOA.	(Griveau et al., 2006)
1.76 mmol/L PTX and 30 min incubation	Using PTX for stimulation of immotile spermatozoa before ICSI, in case of Kartagener's syndrome, the need of invasive interference, such as TESE was reduced.	(Yildirim et al., 2009)
3 mmol/L PTX and 15-min incubation at 37°C	In NOA, using PTX for selecting viable spermatozoa was comparable to hypoosmotic swelling (HOS) test.	(Mangoli, Mangoli, Dandekar, Suri, & Desai, 2011)
50, 100 and 200 μg/ml PTX and then 45-min and 24-, 36- and 48-hr incubation	PTX at 200 μg/ml dose significantly improved sperm viability in oligoasthenozoospermia and mean percentage of live spermatozoa had reduced significantly in all groups through time.	(Ghasemzadeh, Karkon-Shayan, Yousefzadeh, Naghavi-Behzad, & Hamdi, 2016)

TABLE 4 Effectiveness of PTX usage in improving sperm morphology in ART programme

PTX dosage and incubation time	Conclusion	Authors
1 mg/ml (3.6 mmol/L) PTX and 30-min incubation (37°C)	After PTX usage in cases of asthenozoospermia, the percentage of normal sperm morphology and count were within the normal range.	(Khalili et al., 1999)
1 ml of FertiCult Flushing medium + PTX then 8- to 10-min incubation at 37°C	Glass wool filtration technique combined with PTX significantly enhanced normal sperm morphology as compared to the Glass wool filtration technique alone consistent with WHO (2010).	(Kadhim, Mossa, & Selman, 2017)
3.6 mmol/L PTX and 30-min incubation at 37°C	PTX inverted harmful effects of vitrification on sperm motility, but did not induce specific ultrastructural alterations in human vitrified spermatozoa.	(Nabi, Khalili, Talebi, et al., 2017)

7 | PTX AND EMBRYO QUALITY

Preliminary studies have shown that PTX may induce artificial activation and morphological changes in the oocyte, leading to embryo developmental obstruction or teratogenic effects of neonates (Fisher & Gunaga, 1975; Tournaye et al., 1995). However, in a clinical setting, PTX is only present in the prepared sperm sample and the oocytes/embryos are not exposed to culture drops (Hattori et al., 2011). In some instances, the spermatozoa is washed post-exposure to PTX, to further reduce the risk of injection of PTX into the oocyte during ICSI (Griveau, Lobel, Laurent, Michardiere, & Le Lannou, 2006). In general, research about the effect of PTX on human embryo quality and grading is very scarce (Table 6). In these studies, embryo quality was evaluated by means of blastomere number (cleavage rate) and fragmentation percentage and embryos scored as good, fair and poor.

8 | PTX AND ART CLINICAL OUTCOMES

Consequences of PTX treatment in terms of ART results are contradictory (Mehta & Sigman, 2014). Some studies observing no improvement in outcomes (Abdel Raheem et al., 2013; Dimitriadou et al., 1995; Tournaye, Janssens, Verheyen, Devroey, & Van Steirteghem, 1994), while others demonstrating an improvement in fertilisation rates (Table 7). This enhancement could be due to the better selection of viable sperm cells that led to the generation

TABLE 5 Influential role of PTX on sperm DNA integrity

PTX dosage and incubation time	Type of sperm DNA fragmentation assays	Conclusion	Authors
1 mmol/L PTX and 30 min incubation at 37°C, DNA integrity was assessed 6 hr. post-exposure	SCD	Sperm chromatin integrity was not significantly influenced by PTX until 6 hr. post-exposure.	(Asokan et al., 2015)
3.6 mmol/L PTX for 30-min incubation at 37°C	SCD, TUNEL assay, CMA3 staining, Aniline blue staining, Toluidine blue staining	PTX usage did not significantly damage DNA integrity of post-thaw sperm cells in asthenozoospermic patients.	(Nabi, Khalili, Fesahat, et al., 2017)
1.5, 1.25 and 1 mmol/L PTX and 20-min incubation at 37°C	SCD	Application of PTX at 1.5 mmol/L did not affect sperm DNA integrity.	(Dutra, da Silva, Lazzari, Stein, & Filho, 2018)
1 mmol/L PTX and 60-min incubation at 37°C, DNA integrity was assessed 24 and 48 hr. post-exposure	SCD	Insignificant difference in sperm DNA integrity was observed between the PTX and control groups.	(Salian et al., 2019)

TABLE 6 Effectiveness of PTX usage in improving embryo quality in ART programme

PTX dosage and incubation time	Conclusion	Authors
7.2 mmol/L PTX and 10-min incubation at room temperature	Short incubation of spermatozoa to PTX and subsequent sperm washing did not influence early embryo development after ICSI.	(Terriou et al., 2000)
100 mg/5 ml PTX and 5-min incubation at 37°C	Treatment of spermatozoa with PTX increased total numbers of good quality embryos in asthenozoospermic patients.	(Amer et al., 2013)

TABLE 7 Effectiveness of PTX usage in improving the clinical outcomes in ART programme

PTX dosage and incubation time	Conclusion	Authors
1 mg/ml (3.6 mmol/L) PTX and 30- to 40-min incubation	PTX had significant role in improving the fertilisation rate and decreasing the fertilisation failure in certain case of male factor infertility.	(Yovich, 1993)
1 mg/1 ml PTX and 10-min incubation at 37°C	Treatment of spermatozoa with PTX improved the fertilising capacity in patients with acrosome reaction deficiency.	(Tesarik & Mendoza, 1993)
1 mg/ml(3.6 mmol/L) PTX and 30-min incubation at 37°C	PTX improved total number of motile spermatozoa and subsequent fertilising capacity in asthenospermic and oligoasthenospermic samples.	(Tarlatzis et al., 1995)
22 mg of PTX added to 10 ml of Earle's balanced salt solution containing 8% serum albumin	PTX increased the clinical outcomes in couples with male factor infertility and poor fertilisation rates. The study did not propose any rise in teratogenicity or evidence of congenital malformations.	(Rizk et al., 1995)
5 mmol/L PTX and 30-min incubation at 37°C	Treatment of spermatozoa with PTX decreased sperm damage and improved fertilisation rate in IUI or ICSI.	(Esteves, Sharma, Thomas, & Agarwal, 1998)
7.2 mmol/L PTX and 10-min incubation at room temperature	PTX induced motility in immotile epididymal and testicular spermatozoa. The pregnancy and implantation rates per transferred embryo were similar in PTX and control groups.	(Terriou et al., 2000)
1.76 mmol/L PTX and 20-min incubation at 37°C	PTX improved fertilisation rates and the number of embryos per cycle in azoospermia patients.	(Kovacic et al., 2006)
1.5 mmol/L PTX and 10-min incubation	After PTX treatment in NOA, pregnancy and implantation rates were like to those of fresh ejaculated sperm cells.	(Griveau et al., 2006)
1 mg/ml PTX and 10-min incubation	Birth of a healthy infant after insemination of PTX-activated ejaculated immotile spermatozoa from a Kartagener's syndrome case and transfer of a single vitrified warmed blastocyst.	(Hattori et al., 2011)
100 mg/5 ml PTX and 5-min incubation at 37°C	Treatment of spermatozoa with PTX increased fertilisation rate and embryos transferred in asthenozoospermic patients.	(Amer et al., 2013)
5 µl PTX and 10- to 20-min incubation at 37°C	PTX treatment did not enhance hostile obstetric and neonatal outcomes.	(Navas et al., 2017)

of high developmental potential embryos (Griveau et al., 2006). Available data about PTX safety on offspring are very scant, and based on some studies, its application is easy and quick. Also, to date, no malformations have been reported in newborns conceived using PTX-activated spermatozoa (Griveau et al., 2006). Recently, Navas and associates followed the newborns after ICSI using sperm cells exposed with PTX (Navas et al., 2017). They classified newborn malformations according to the International Classification of Diseases and health-related problems ICD-10 (ICD-Code, 2007) involving inherited malformations, distortions and chromosomal abnormalities. In their study, the frequency of inherited malformations (3.3%) was not significantly higher than that detected in IVF offspring and in the general European population reported by EUROCAT. While several epigenetic syndromes have been linked to ICSI and IVF, including Beckwith–Wiedemann syndrome, Angelman syndrome and retinoblastomas (Gicquel et al., 2003), no additional epigenetic syndromes were introduced in PTX-ICSI births. Therefore, it seems that PTX treatment does not enhance hostile obstetric and neonatal outcomes, though the cohort was small and only newborn data were accumulated. Furthermore, this study needed a long-term follow-up for the majority of the children (Navas et al., 2017).

9 | CONCLUSION

Although the present review shows interest in the use of PTX as a sperm movement enhancer, there is a difference in the response of spermatozoa to PTX in normozoospermic and abnormal sperm samples. Therefore, it seems that a diagnosis of the individual response to PTX such as the acrosome reaction to ionophore challenge test (ARIC) is necessary before routine use in an ART programme. The majority of researchers utilised a concentration of 3–5 mmol/L of PTX and 10- to 30-min incubation duration. PTX requires a threshold concentration to act as sperm motility enhancer, but high concentration and a long exposure time may have negative effects on sperm parameters and membrane integrity. In conclusion, with sperm motion is elevated after PTX treatment, the long-term consequences on embryo quality and clinical parameters are not yet sufficiently recorded. Use of new embryonic examination methods, including 'omics' and time-lapse imaging techniques maybe helpful for evaluation of embryos derived from PTX-treated spermatozoa. Therefore, more examinations with larger sample sizes and extended time of investigation are required to endorse the PTX's safety in assisted male reproduction.

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