Antidiabetic therapies and male reproductive function: where do we stand?

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Abstract

Diabetes mellitus has been increasing at alarming rates in recent years, thus jeopardizing human health worldwide. Several antidiabetic drugs have been introduced in the market to manage glycemic levels, and proven effective in avoiding, minimizing or preventing the appearance or development of diabetes mellitus-related complications. However, and despite the established association between such pathology and male reproductive dysfunction, the influence of these therapeutic interventions on such topics have been scarcely explored. Importantly, this pathology may contribute toward the global decline in male fertility, giving the increasing preponderance of diabetes mellitus in young men at their reproductive age. Therefore, it is mandatory that the reproductive health of diabetic individuals is maintained during the antidiabetic treatment. With this in mind, we have gathered the available information and made a critical analysis regarding the effects of several antidiabetic drugs on male reproductive function. Unlike insulin, which has a clear and fundamental role on male reproductive function, the other antidiabetic therapies' effects at this level seem incoherent. In fact, studies are highly controversial possibly due to the different experimental study approaches, which, in our opinion, suggests caution when it comes to prescribing such drugs to young diabetic patients. Overall, much is still to be determined and further studies are needed to clarify the safety of these antidiabetic strategies on male reproductive system. Aspects such as the effects of insulin levels variations, consequent of insulin therapy, as well as what will be the impact of the side effect hypoglycemia, common to several therapeutic strategies discussed, on the male reproductive system are still to be addressed.

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Diabetes, antidiabetic drugs and male reproductive function

Diabetes mellitus is a global epidemic that currently affects over 400 million adults worldwide and it is predicted to raise to over 600 million in 2040 (IDF 2015, WHO 2016). This is a complex metabolic systemic disease characterized by hyperglycemia, that results from impaired insulin action and/or secretion, being the main cause of micro and macro vascular pathologies and one of the leading causes of morbidity and mortality (Alberti & Zimmet 1998, Emilien *et al.* 1999, Roglic *et al.* 2005), contributing to 5 million annual deaths worldwide, ultimately having a devastating human, social and economic impact (IDF 2015, WHO 2016).

The principal forms of this disease are type 1 and type 2, the first being caused by the immune destruction of β -pancreatic cells and lack of insulin production while the second, the more prevalent form, is characterized

by insulin resistance and inappropriate insulin secretory response (Alberti & Zimmet 1998, ADA 2016b). Although genetic and independent accelerating factors might have a role, hyperglycemia is recognized as one of the main perpetrators of the disease-induced complications, through the activation of a variety of damage pathways that seem to be triggered by mitochondrial superoxide overproduction (TDC&CTRG 1993, Brownlee 2005), emphasizing the role of oxidative stress in the development of clinical complications associated with the disease (Ahmed 2005, Pitocco *et al.* 2010). However, due the complexity of the disease, we cannot exclude that other players might also be involved in the pathophysiology of the disease (Greenfield & Campbell 2006, Muoio & Newgard 2008).

Not surprisingly, all the levels of the male reproductive system seem to be a target for the deleterious effects of diabetes. In fact, this degenerative disease was described to affect not only sperm quality and function

(Bartak *et al.* 1975, Padron *et al.* 1984, Handelsman *et al.* 1985, Vignon *et al.* 1991, Baccetti *et al.* 2002, Soudamani *et al.* 2005, Amaral *et al.* 2006, Scarano *et al.* 2006, Agbaje *et al.* 2007, Delfino *et al.* 2007, Shrilatha & Muralidhara 2007a, Rama Raju *et al.* 2012, Roessner *et al.* 2012, Schoeller *et al.* 2012a, Mangoli *et al.* 2013) but also the hypothalamus–pituitary–gonadal axis (HPG) (Benitez & Perez Diaz 1985, Seethalakshmi *et al.* 1987, Sudha *et al.* 2000, Baccetti *et al.* 2002, Lopez-Alvarenga *et al.* 2002, Ballester *et al.* 2004, Kim & Moley 2008, Schoeller *et al.* 2012b), the testicular function and spermatogenesis (Cameron *et al.* 1985, Gondos & Bevier 1995, Sanguinetti *et al.* 1995, Sainio-Pollanen *et al.* 1997, Ballester *et al.* 2004, Arikawe *et al.* 2006, Amaral *et al.* 2009, Kianifard *et al.* 2012, Schoeller *et al.* 2012b, Wankeu-Nya *et al.* 2013, Kyathanahalli *et al.* 2014, Tavares *et al.* 2016), the accessory organs (epididymis, prostate and seminal vesicles) (Murray *et al.* 1981, Fukumoto *et al.* 1993, Ikeda *et al.* 2000, Saito *et al.* 2000, Soudamani *et al.* 2005, Morrison *et al.* 2006, Singh *et al.* 2009, La Vignera *et al.* 2011) and both erectile and ejaculatory function (Penson *et al.* 2009). Nonetheless, some controversy exists in the literature regarding this topic, and there are also some studies that reported no significant modifications or changes only in some of the aspects (Niven *et al.* 1995, Navarro-Casado *et al.* 2010, La Vignera *et al.* 2015). These different reports might be related to aspects that varied among studies, such as duration of the disease, type of glycemic control, patient and animal model selection, experimental design and technical approaches.

Although it is not entirely understood how the reproductive impairment in males is orchestrated, recent reports showed that fertility in diabetic males and animal models is impaired (Ballester *et al.* 2004, Kim & Moley 2008, Bener *et al.* 2009, La Vignera *et al.* 2009a) and that diabetic patients frequently seek for fertility treatments, pregnancy rates being reduced in couples in whom the male is diabetic (Mulholland *et al.* 2011). Such evidence further supports that something is wrong in the reproductive potential of these patients and that alterations at several levels of the male reproductive system might have happened. Additionally, this also stresses the importance of monitoring diabetic patients' reproductive health, mainly because the epidemic rise of the disease is affecting more and more men, and worryingly, at the acme of their reproductive phase (Agbaje *et al.* 2007), which might compromise the likelihood of fathering a child.

It is widely accepted that good management of diabetes, namely the glycemic control, is a key component to minimize, avoid or prevent diabetic complications (TDC&CTRG 1993, El-Kaissi & Sherbeeni 2011, ADA 2016c). At the male reproductive level, this is also a quite pertinent issue considering that: (1) glucose was reported to be at higher levels in the semen of diabetic patients (Padron *et al.* 1984) raising the question

whether hyperglycemia, besides the systemic effects, may also contribute toward the alterations observed in diabetic patients' sperm; (2) a poor metabolic/glycemic control was negatively associated not only with sperm parameters (Padron *et al.* 1984, La Vignera *et al.* 2009b, 2015, Roessner *et al.* 2012) but also with other reproductive features (Ahn *et al.* 2007, La Vignera *et al.* 2011, Choi *et al.* 2015), though some studies did not find any relation (Niven *et al.* 1995). In this way, another essential question arises: how will the antidiabetic drugs impact the male reproductive function? Though habitually overlooked, considering the epidemic raise of diabetes and its high prevalence in young males, this constitutes a crucial point, as it is important to guarantee the reproductive health of diabetic individuals during antidiabetic treatments. To address this pertinent subject, we critically analyzed the existing literature focused on the effects of several antidiabetic strategies on various aspects of the male reproductive system (Table 1).

Currently, there is a large variety of antidiabetic therapies available, which although varying in the mechanisms of action, efficacy, side effects and cost, have the same purpose, that is to control blood glucose levels (El-Kaissi & Sherbeeni 2011, ADA 2016d). Although for type 1 diabetic patients, insulin therapy is indispensable, the basis of primary management for type 2 patients entails lifestyle modifications along with metformin treatment (El-Kaissi & Sherbeeni 2011, ADA 2016d). Moreover, for type 2 diabetes, other treatments exist, such as sulphonylureas, meglitinides, insulin and thiazolidinediones/glitazones. However, as these therapeutic options are associated with weight gain and/or hypoglycemia and increase risk of cardiac and kidney disease (El-Kaissi & Sherbeeni 2011, ADA 2016d, Skiros *et al.* 2016), alternative therapies were developed to overcome these problems as it is the case of α -glucosidase inhibitors, glucagon-like peptide-1 agonists, dipeptidyl peptidase-IV (DPP-4) inhibitors, sodium-glucose transporter 2 (SGLT2) inhibitors, amylin analogues and bile acid sequestrants. These pharmaceuticals can also be used in combination in some cases (El-Kaissi & Sherbeeni 2011, ADA 2016d). However, the optimal approach form of glycemic control should be patient-tailored, having in consideration the existence of comorbidities and side effects (El-Kaissi & Sherbeeni 2011, ADA 2016a). Nonetheless, their impact on male reproductive function is not fully disclosed.

Insulin

Being secreted by the β -pancreatic cells after nutrient ingestion, insulin constitutes the earliest introduced treatment for diabetes, discovered back in the 1920s (Shabanpoor *et al.* 2009, El-Kaissi & Sherbeeni 2011, Zhang & Liu 2014). Worth mentioning, more is known about the effects of this drug on the male reproductive function, mainly because this hormone

Table 1 Effects of antidiabetic agents on the male reproductive system in a diabetic context.

Antidiabetic agent/duration of the treatment			Effects on male reproductive system		Reference
Antidiabetic agent/duration of the treatment	Species/model/age	Type of study	Level	Observations	
Insulin implants/8 weeks	Akita mice/8–12 weeks old	<i>In vivo</i>	Overall	Fertility impairment was rescued.	Schoeller <i>et al.</i> (2012a)
			Pre-testicular	The levels of serum LH were normalized after treatment.	
			Testicular	Testis weight was restored, as well as the testicular cells population.	
				The levels of serum testosterone were normalized after treatment.	
			Post-testicular	Sperm motility has increased to levels similar to the wild type.	
Short acting neutral insulin/zinc suspension/6 days	STZ-induced diabetic Wistar rats/mature	<i>In vivo</i>	Pre-testicular	All parameters of pulsatile LH secretion were reversed to control values.	Dong <i>et al.</i> (1991)
Beef/pork insulin/4 weeks	STZ-induced diabetic sheep/12-week-old	<i>In vivo</i>	Pre-testicular	LH pulse frequency was maintained at levels similar to non-diabetic controls.	Bucholtz <i>et al.</i> (2000)
				Re-supplementation restored the decrease in LH pulse frequency caused by insulin withdrawal.	
Long acting human recombinant insulin/5 days	STZ-induced diabetic sheep/1 year old	<i>In vivo</i>	Pre-testicular	Central insulin infusion increased LH pulse frequency when compared to saline treated animals, but in a less extent than peripheral treatment.	Tanaka <i>et al.</i> (2000)
Insulin/20 days	STZ-induced diabetic Wistar rats/30 days old (prepubertal) and 50 days old (pubertal)	<i>In vivo</i>	Pre-testicular	Serum LH levels were restored in prepubertal and pubertal rats.	Sudha <i>et al.</i> (2000)
				Serum FSH and prolactin levels were not restored in prepubertal rats.	
			Testicular	The activity of steroidogenic enzymes in Leydig cells isolated from diabetic animals was restored to control levels.	
		<i>In vitro</i>		Serum testosterone levels were restored in prepubertal and pubertal rats.	
Bovine insulin Betasint/3 h 30 min	Diabetic Wistar rats' Sertoli and Leydig cells/adult	<i>In vitro</i>	Testicular	Arachidonic acid synthesis was partly restored.	Hurtado de Catalfo and De Gomez Dumm (1998)
Porcine insulin/20 days	STZ-induced diabetic Wistar rats/40 days old	<i>In vivo</i>	Post-testicular	The weight, the epithelium atrophic changes and the luminal diameter of the epididymis were partially recovered.	Soudamani <i>et al.</i> (2005)
Porcine insulin/20 days	STZ-induced diabetic Wistar rats/40 days old	<i>In vivo</i>	Post-testicular	The decrease in epididymal testosterone and ABP level was partially or completely prevented by the treatment.	Singh <i>et al.</i> (2009)
				The decrease in epididymal secretory function was partially or completely prevented by the treatment.	
				Sperm motility and fertilizing capacity were restored.	

(Continued)

Table 1 Continued.

Antidiabetic agent/duration of the treatment	Species/model/age	Type of study	Effects on male reproductive system		Reference
			Level	Observations	
Protamine zinc insulin/8 weeks	STZ-induced diabetic Sprague–Dawley rats/55–56 days old	<i>In vivo</i>	Post-testicular	In the prostate, the levels of androgen and estrogen receptors were partially or completely normalized and the biochemical profiles improved.	Suthagar <i>et al.</i> (2009)
Protamine zinc insulin/42 days	STZ-induced diabetic Sprague–Dawley rats/adult	<i>In vivo</i>	Pre-testicular	The levels of serum prolactin were normalized after the treatment.	Steger & Kienast (1990)
Insulin/4–8 weeks	STZ-induced diabetic Wistar rats/adult	<i>In vivo</i>	Testicular	The levels of serum testosterone were normalized after the treatment.	Yonezawa <i>et al.</i> (2009)
NPH, human insulin isophane suspension (intermediate acting)/8 weeks	STZ-induced diabetic Sprague–Dawley rats/6 months old	<i>In vivo</i>	Post-testicular	Deficits in mount, intromission and ejaculatory behaviors were prevented when the treatment was administered continuously.	
Intermediate acting insulin/6 weeks	STZ-induced diabetic Sprague–Dawley rats/8 weeks old	<i>In vivo</i>	Post-testicular	Ejaculatory dysfunction was prevented when insulin was administered at an early stage. Intracavernous pressure decrease was restored. Recovered anti-apoptotic protein expression in rat crura.	Yamanaka <i>et al.</i> (2003)
Metformin (biguanides)/6 weeks	Alloxan-induced diabetic Wistar rats/9–10 weeks old	<i>In vivo</i>	Post-testicular	Erectile dysfunction and histological changes were partially restored after treatment.	Wang <i>et al.</i> (2014)
			Pre-testicular	Serum LH, FSH and prolactin levels did not change after the treatment.	Adaramoye & Lawal (2014)
			Testicular	Testis relative weight was similar between the treated condition and the diabetic control but lower than the non-diabetic group. Testicular protein and sialic acid contents, as well as, testicular GST, GSH, GSH-Px, catalase and SOD activities were not improved by the treatment and remained lower when compared to the non-diabetic control. Testicular lipid peroxidation was not attenuated after treatment and remained increased when compared to the non-diabetic condition. Serum testosterone levels did not change after the treatment.	
			Post-testicular	Sperm motility increased to levels even similar to the non-diabetic controls. Epididymal sperm count and total sperm abnormalities did not alter after the treatment and remained decreased and increased, respectively, in relation to the non-diabetic control. Sperm live/dead ratio did not change between the treated and untreated conditions nor in comparison to the non-diabetic control.	

Metformin (biguanides)/35 days	STZ-induced diabetic Sprague-Dawley rats/adult	<i>In vivo</i>	Testicular	Diabetes-induced testicular pathological changes were ameliorated. Testicular IGF-1 protein and mRNA expression increased after treatment but mRNA expression decreased in relation to non-diabetic animals. Normalization of testicular IGF-1 protein in comparison with the non-diabetic controls. Testicular GH protein expression decreased, reaching a level similar to the one found in non-diabetic animals. Testicular GH mRNA expression decreased after treatment, but increased in relation to the non-diabetic controls. Serum testosterone levels increased after the treatment but still decreased in comparison with the non-diabetic controls.	Song et al. (2015)
Metformin (biguanides)/8 weeks	STZ-induced adult diabetic Wistar rats/10–12 weeks old	<i>In vivo</i>	Post-testicular	Diabetes-induced biochemical alterations, typical of oxidative stress (lipid peroxidation and GSH levels) were normalized after treatment. Sperm quality was restored following treatment at the highest dose.	Attia et al. (2009)
Metformin (biguanides)/6 weeks	STZ-induced diabetic Sprague-Dawley rats/3 months old	<i>In vivo</i>	Testicular	The treatment provoked the smallest histological modifications on testis, epididymis and seminal vesicle structures when compared to pioglitazone and the DPP-4 inhibitor sitagliptin. Testis, epididymis and seminal vesicle weight decrease was prevented by the treatment. Serum testosterone levels increased with the treatment, reaching values similar to the non-diabetic animals.	Ayub et al. (2015)
Metformin (biguanides)/50 h	Wistar rat primary Sertoli cells cultures in medium containing high glucose levels	<i>In vitro</i>	Testicular	Reduced mRNA and protein levels of PFK 1, MCT4, GLUT1 and GLUT3. LDH mRNA and protein levels did not change, but LDH activity was increased. Increased alanine and lactate production. There was a decrease of abnormal sperm shapes after treatment.	Alves et al. (2014)
Glibenclamide (sulfonylureas)/4 weeks	NA-STZ-induced diabetic Wistar rats/8 weeks old	<i>In vivo</i>	Post-testicular	Testis weight increased.	Rabbani et al. (2010b)
Gliclazide (sulfonylureas)/55 days	STZ-induced diabetic Wistar rat/adult	<i>In vivo</i>	Testicular	Testicular 3 β -HSD and 17 β -HSD enzyme activity levels and testosterone levels increased. The diameter of seminiferous tubules and epithelial layer thickness increased.	Nelli et al. (2013)
			Post-testicular	Epididymis, seminal vesicle and prostate relative weight increased. Sperm count, motility, viability, and HOS tail coiled sperm increased.	
Glimepiride (sulfonylureas)/4 weeks	NA-STZ-induced diabetic Wistar rats/8 weeks old	<i>In vivo</i>	Post-testicular	Sperm shape abnormalities decreased. Sperm count increased.	Rabbani et al. (2009)

(Continued)

Table 1 Continued.

Antidiabetic agent/duration of the treatment	Species/model/age	Type of study	Effects on male reproductive system		Reference
			Level	Observations	
Rosiglitazone (glitazones)/8 weeks	Diabetic ZDF (fa/fa) rats/6 weeks old	<i>In vivo</i>	Testicular	The detachment and disorganization of germ cells was reversed.	Mansour et al. (2009)
Pioglitazone (glitazones)/4 and 8 weeks	Alloxan-induced hyperglycemic rabbits/adult	<i>In vivo</i>	Overall	Serum anti-oxidative enzymes (Cu,Zn-SOD; CAT; GSH-Px; GSSG-R) activity ameliorated.	Gumieniczek et al. (2008, 2011)
			Testicular	Lipid and protein oxidation was reduced. Level of myeloperoxidase enzyme was reduced. Aconitase enzyme activity normalized.	
Pioglitazone (glitazones)/6 weeks	STZ-induced diabetic Sprague-Dawley rats/3 months old	<i>In vivo</i>	Overall	Histopathological changes in testis, epididymis and seminal vesicles were maintained or worsened.	Ayuob et al. (2015)
			Testicular	Serum lipid peroxidation decreased. The weight of the testis was ameliorated. Serum testosterone increased. Testicular ER α and ER β immunoeexpression decreased.	
Pioglitazone (glitazones)/24 h	Human Sertoli cells cultured with high glucose concentration	<i>In vitro</i>	Post-testicular	The weight of the epididymis decreased. ER β and AR immunoreactivity in epididymis and seminal vesicles was decreased.	
Pioglitazone (glitazones)/4 weeks	NA-STZ-induced diabetic Wistar rats/8 weeks old	<i>In vivo</i>	Testicular	Glycolytic efficiency was increased and lactate production was stimulated.	Meneses et al. (2016)
			Overall	Lipid peroxidation was reduced. Serum GSH levels increased. Serum antioxidant enzyme GPx increased. Sperm count was increased.	Rabbani et al. (2010a)
Pioglitazone (glitazones)/22 weeks	Diabetic Zucker (fa/fa) rat/6 weeks old	<i>In vivo</i>	Post-testicular	Sperm shape abnormality decreased.	
Sitagliptin (DPP-4 inhibitors)/3 months	Diabetic man/39 year old	<i>In vivo</i> Case Report	Post-testicular	Penile corporal fibrosis, oxidative stress and corporal veno-occlusive dysfunction (CVOO) were ameliorated.	Kovanez et al. (2006)
			Post-testicular	Semen volume was decreased. Sperm concentration and motility were also decreased.	Hilbi et al. (2011)
Sitagliptin (DPP-4 inhibitors)/6 weeks	STZ-induced diabetic Sprague-Dawley rats/3 months old	<i>In vivo</i>	Testicular	Serum free testosterone levels decreased. Pathological changes caused by diabetes in the structure and weight of the testis were slightly ameliorated.	Ayuob et al. (2015)
			Post-testicular	Pathogenic changes caused by diabetes in the structure and weight of the epididymis and seminal vesicle were reduced. The expression of estrogen and androgen receptors in the corpus and cauda of the epididymis decreased.	

Acarbose (alpha-glucosidase inhibitors)/7 days	STZ-induced diabetic Wistar rats/adult	In vivo	Testicular	Spermatocyte, spermatogonial cell, and sertoly cell number increased after treatment.	Sadeghi et al. (2013)
Exenatide (GLP-1 analogues)/8 weeks	Obese C57BL/6j mice/7–8 weeks old	In vivo	Post-testicular	Sperm motility increased. Sperm mitochondrial membrane potential increased.	Zhang et al. (2015)
Liraglutide (GLP-1 analogues)/5 months	Diabetic man/35 year old	In vivo	Post-testicular	Sperm DNA damage decreased. Sperm production was interrupted.	Fontoura et al. (2014)
Dapagliflozin (SGLT2 inhibitors)/24 weeks	Diabetic man/≥ 18 years old	In vivo	Overall	Developed urogenital tract infections.	Ji et al. (2014)

Due to the extensive amount of data regarding the insulin effects on male reproductive function, only studies after 1990 are presented in this table. AA, arachidonic acid; ABP, androgen binding protein; AMP:ATP, adenosine monophosphate:adenosine triphosphate ratio; AMPK, AMP-activated protein kinase; AR, androgen receptor; BW, body weight; CAT, catalase; ER, estrogen receptor; FSH, follicle-stimulating hormone; GH, growth hormone; GLUT1, glucose transporter 1; GLUT3, glucose transporter 3; GnRH, gonadotrophin-releasing hormone; GSH, glutathione; GSH-Px or GPx, glutathione peroxidase; GSSG-R, glutathione reductase; GST, glutathione S-transferase; hCG, human chorionic gonadotropin; IGF-1, insulin growth factor 1; LDH, lactate dehydrogenase; LH, luteinizing hormone; MCT4, monocarboxylate transporter 4; mTOR, mammalian target of rapamycin; NA, nicotinamide; PFK1, phosphofructokinase 1; PPAR, peroxisome proliferator-activated receptor; SOD, superoxide dismutase; STZ, streptozotocin; TGF, transforming growth factor.

is normally present in the body and is crucial for physiological homeostasis.

Though insulin therapy is imperative for patients with type 1 diabetes and might also be used by some patients with type 2 diabetes in more advanced stages of the disease, this therapy has been frequently associated with the occurrence of hypoglycemia that, if not managed appropriately, might have severe consequences that can ultimately lead to mortality (Cryer 2008).

The insulin signaling network is rather complex and its activation is dependent on the insulin binding to its receptors and recruitment of insulin receptor substrate, followed by the activation of two key signaling pathways, the phosphatidylinositol-3-kinase (PI3K) pathway and the mitogen-activated protein kinase pathway, through which insulin will accomplish its functions regulating a panoply of processes (Zhang & Liu 2014). Besides the role of insulin in glucose disposal, this hormone is also involved in the modulation of other processes such as cellular growth, proliferation differentiation and survival (Zhang & Liu 2014).

Importantly, as the energy stores need to be managed properly to assure physiological function, a close relationship exists between insulin signaling and fertility (Porte et al. 2005, Mauvais-Jarvis 2016), raising the question on how insulin-deregulated action and levels oscillation occurring not only during the time course of the disease (hyperinsulinemia/hypoinsulinemia), but also during therapy (hyperinsulinemia), will influence male reproductive function.

Insulin and male reproductive function

The interest on the insulin effects on the male reproductive function is well mirrored by the numerous reports found in the literature. In fact, insulin action is extended to all the levels of the male reproductive system, from the HPG to the testis, also having a role in male accessory organs, erectile and ejaculatory function and ultimately on sperm, as we will discuss in the following sections.

Insulin was shown to interfere with the HPG axis, stimulating LH and FSH release in pituitary cell cultures (Adashi et al. 1981) and GnRH secretion and expression in hypothalamic neurons in culture (Burcelin et al. 2003). Furthermore, the brain-specific KO for insulin receptor resulted in male mice with decreased fertility. Alterations such as reduction in offspring, impaired spermatogenesis, decline in epididymal sperm content and decrease in Leydig cells population and in serum LH have been reported, suggesting a role for insulin in the brain hormonal output, and consequences of its absence at the testicular level, compromising spermatogenesis' outcome (Bruning et al. 2000).

However, insulin might also act directly in the testis. In fact, insulin receptors as well as insulin expression has been described in the testis, pointing to an important role of insulin in spermatogenesis regulation

(Saucier *et al.* 1981, Gomez *et al.* 2009). It is therefore logical to hypothesize that the insulin variations that occur during diabetes might affect insulin signaling at the testis level, potentially affecting spermatogenesis.

Although the cellular complexity of the testis might complicate the understanding of insulin signaling in this organ, evidence is now available regarding almost all the cell types that participate in the spermatogenesis, namely germ and the somatic Sertoli and Leydig cells.

At the Sertoli cell level, *in vitro* studies have shown that insulin-stimulated androgen-binding protein (ABP) secretion, which is an indicator of Sertoli cell function (Karl & Griswold 1980). Additionally, insulin was reported to have positive effects on DNA and protein synthesis (Borland *et al.* 1984) and on lactate (Borland *et al.* 1984, Oonk *et al.* 1985) and transferrin secretion (Skinner & Griswold 1982) in cultured rat Sertoli cells. Later on, insulin receptors were identified in Sertoli cells (Oonk & Grootegoed 1987), suggesting that the effects of insulin on these cells were receptor-mediated and that insulin has a determinant role on Sertoli cells metabolism and function, which might have consequences in terms of spermatogenesis. In fact, subsequent studies support this hypothesis with several metabolic alterations being reported when human Sertoli cells were cultured in the absence of insulin (Alves *et al.* 2012, Oliveira *et al.* 2012). Moreover, the authors further investigated this issue describing that insulin deprivation led to a deregulation in the apoptotic signaling in rat Sertoli cells (Dias *et al.* 2013). The authors suggested that these alterations might compromise spermatogenesis and contribute to the adverse effects of type 1 diabetes, and its typical insulin deficiency, on male reproductive system (Alves *et al.* 2012, Oliveira *et al.* 2012, Dias *et al.* 2013).

Insulin receptors are also reported in Leydig cells (Lin *et al.* 1986), and insulin was described to have a role on these cells' growth and proliferation (Khan *et al.* 1992). Furthermore, insulin was observed to increase testosterone production in primary Leydig cell cultures (Lin *et al.* 1986). Hence, insulin might exert its effects directly on Leydig cells or through its action on LH that will further regulate Leydig cell function.

Finally, there are also data to support a role of insulin on germ cells, promoting rat spermatogonial DNA synthesis and new spermatogonia differentiation, corroborating its important role on spermatogenesis (Soder *et al.* 1992, Nakayama *et al.* 1999). However, insulin's role on the male reproductive function is not limited to the HPG and testis, and soon a role of insulin on sperm was recognized.

Early in the 1970s, insulin was described as an important regulator of sperm metabolism, as it was observed that *in vitro* addition of insulin to human sperm increased motility and glucose utilization, primarily through the stimulation of pentose pathway and Krebs

cycle (Hicks *et al.* 1973). Nonetheless, the insulin's role on sperm glucose metabolism modulation was questioned by divergent observations reported some years later (Gorus & Pipeleers 1986).

Considering that, in the female follicular fluid, insulin levels peak at the ovulation time, Silvestroni and coworkers investigated the effects of insulin on human sperm and have shown that this hormone acts at the acrosome and sperm plasma membrane, suggesting the involvement of insulin in the acrosome reaction, a process that allows sperm–oocyte binding (Silvestroni *et al.* 1992). In a further study regarding the effects of several hormones on rat sperm motility, intratesticular injection of insulin was shown to cause a decrease in vas deferens' sperm motility. However, when sperm were removed from the vas deferens and incubated in phosphate buffer saline *in vitro*, the percentage of motile sperm was observed to increase, which have led the authors to suggest that these divergent effects might be due to physiological aspects of the duct system that maintain the motility down to save energy that might be used in other processes (Sliwa 1994).

Some years later, important observations were made by Aquila and coworkers reporting that insulin is expressed and secreted in human ejaculated sperm. Furthermore, they described that both insulin secretion and glucose metabolism were regulated by insulin in an autocrine fashion and that sperm insulin secretion might have a role in capacitation, a process that confers sperm fertilizing competence. These were significant findings as they suggest that insulin might regulate sperm glucose metabolism depending on the different circumstances and needs, independently of systemic insulin (Aquila *et al.* 2005) and suggesting another possible way by which diabetes can interfere with sperm function (Schoeller *et al.* 2012b).

These results were later corroborated in pigs with the identification of insulin receptors in sperm and the description of an additional role of insulin in the acrosome reaction (Carpino *et al.* 2010). Accordingly, other study has reported positive effects of insulin not only on human sperm motility but also on the acrosome reaction induction and nitric oxide production, validating the importance of this hormone in sperm fertilizing ability (Lampiao & du Plessis 2008).

Finally, KO studies focused on insulin superfamily members (Baker *et al.* 1996, Burnicka-Turek *et al.* 2012), their receptors (Nef *et al.* 2003) or on intermediaries of insulin signaling pathway (Griffith *et al.* 2013), provided further data supporting the importance of insulin signaling pathway on the male reproductive function, namely on testicular aspects and sperm characteristics (Baker *et al.* 1996, Burnicka-Turek *et al.* 2012, Griffith *et al.* 2013) as well as on male sex determination during development (Nef *et al.* 2003) are present.

Data from studies in diabetic individuals/conditions

There are several details that remain to be clarified when it comes to the mechanisms responsible for the alterations in diabetics' reproductive function. Though hyperglycemia and associated oxidative stress are likely candidates to explain some of the alterations (Agbaje *et al.* 2007, Shrilatha & Muralidhara 2007a,b, La Vignera *et al.* 2009b, Rama Raju *et al.* 2012), the deregulation in insulin signaling pathway during diabetes cannot be excluded from this network of damage. In fact, having in mind the findings discussed so far, it seems reasonable to hypothesize two ways by which insulin might impact the male reproductive function: through the regulation of glucose metabolism, avoiding the negative impact of hyperglycemia, or inducing alterations due to hypoglycemia or by having a direct role at the cellular level, activating important signaling pathways.

Studies in diabetic individuals or in diabetic conditions, provided important insights concerning the importance of insulin (its absence or excess) to the male reproductive system, with several studies reporting that the reproductive changes found in diabetic individuals could be reverted or attenuated by insulin treatment (Seethalakshmi *et al.* 1987, Gondos & Bevier 1995, Ikeda *et al.* 2000, Yamanaka *et al.* 2003, Kim & Moley 2008, Singh *et al.* 2009, Yonezawa *et al.* 2009, Schoeller *et al.* 2012a).

Due to the extensive data available, we will focus on 3 main levels: pre-testicular, testicular and post-testicular.

Pre-testicular level:

There is a wide disparity regarding the effects of insulin treatment on gonadotrophin levels in diabetic individuals with reports ranging from a total recovery of both LH and FSH (Seethalakshmi *et al.* 1987), to the recovery of just one (Perez Diaz *et al.* 1982, Hutson *et al.* 1983, Benitez & Perez Diaz 1985, Sudha *et al.* 2000) or even the absence of effects (Steger & Kienast 1990), suggesting that testicular function regulation is the result of a complex process in which several players are involved.

Other studies have explored these aspects in more detail, and insulin treatment was observed to totally reverse all parameters of pulsatile LH secretion, which were reduced in diabetic rats and, at least partially, due to a decreased responsiveness of the pituitary to GnRH (Dong *et al.* 1991).

In a different report, Bucholtz and collaborators have found that LH secretion was reduced in developing diabetic sheep with hypoinsulinemia and that this effect was potentiated by sex steroids (Bucholtz *et al.* 2000). Yet, insulin treatment was observed to restore LH pulse frequency (Bucholtz *et al.* 2000). In a follow-up study, the authors observed that central insulin infusion in diabetic sheep increased the cerebrospinal fluid concentration of this hormone, maintaining it at levels similar to those observed after peripheral insulin administration and not altering peripheral insulin or glucose levels. Furthermore, central insulin infusion was observed to increase LH pulse frequency, although in a lower extension than the peripheral

one, suggesting that central insulin has also a role in pulsatile GnRH secretion regulation (Tanaka *et al.* 2000).

Aiming to clarify if the insulin action is dependent on the sexual maturation phase in which diabetes is detected, a study was conducted in both prepubertal and pubertal diabetic rats (Sudha *et al.* 2000). Insulin was observed to restore serum LH and testosterone levels in both groups, highlighting the regulatory role of insulin in gonadotrophin secretion and on Leydig cell steroidogenic ability acquisition during sexual maturation. Nonetheless, FSH and prolactin levels were not restored by insulin on prepubertal rats, suggesting that the initiation of diabetes before puberty impairs the functional maturation of the pituitary-testicular axis in a different fashion and that insulin regulates the pituitary differently, depending on the stage of development (Sudha *et al.* 2000).

Testicular level:

There are several studies that provide evidence for the direct effect of hyperglycemia and/or insulin deficiency on Leydig cells. In early experiments, it was shown that untreated diabetes caused a reduction in serum LH, testosterone levels and steroidogenic activity and that these parameters were restored by insulin treatment (Tesone *et al.* 1976, Murray *et al.* 1981, Perez Diaz *et al.* 1982, Benitez & Perez Diaz 1985), raising the question on whether the effects of insulin are directly in the testis or induced through alterations in LH secretion. Furthermore, it was observed that Leydig cells' LH receptors were decreased in diabetic rats, though the binding capacity was restored after insulin administration (Charreau *et al.* 1978). The authors suggested that this alteration might be behind the altered responsiveness of diabetic Leydig cells to LH (Charreau *et al.* 1978). In addition, Paz and Homonnai reported a reduced number of Leydig cells and testosterone secretion in testis of diabetic animals (Paz & Homonnai 1979). However, in this case, insulin treatment presented no beneficial effects in terms of Leydig cell function (Paz & Homonnai 1979).

In another study, insulin has also been shown to restore the ultrastructure of diabetic rats' Leydig cells, including the accumulation of lipid droplets, reduction of smooth endoplasmic reticulum and presence of intracellular bodies (Orth *et al.* 1979). Therefore, the authors suggested that the reduced steroidogenesis commonly observed in diabetic rats might be, at least in part, due to alterations in Leydig cell ultrastructure and function induced by insulin deficiency.

Tremblay's team, after showing that pituitary LH regulates testicular insulin receptors, measured insulin-binding parameters in testicular membranes of diabetic rats to clarify if the observed alterations in Leydig cell function were due to effects mediated by LH or to a direct effect of altered LH levels (Tremblay *et al.* 1985). The authors observed a decrease in insulin binding in Leydig cell from diabetic rats. As both LH and insulin receptor are dependent on pituitary LH, whose release is also impaired in diabetes, the authors suggested that LH is a key factor to explain the detrimental effects on Leydig cells, including decreased androgen levels and alterations in the reproductive organs (Tremblay *et al.* 1985) and that both LH and insulin receptors, under the influence of LH are needed to the correct the function of Leydig cells.

In a similar study, Ballester and coworkers have shown that insulin and IGF-1 receptors content in the testis of diabetic rats were decreased, mainly in the interstitial tissue (Ballester *et al.* 2004). Additionally, they observed reduced LH levels and almost no insulin in the serum, which have led them to suggest that this might have contributed to a loss of stimulation of androgen synthesis and cell proliferation, in accordance with the observed decrease in Leydig cell number and function. Therefore, the authors proposed that the alterations in Leydig cells can be a consequence of either an effect of hypoinsulinemia on LH levels or the joint effects of decreased LH and insulin levels on Leydig cells (Ballester *et al.* 2004).

In a different study, following the observation of altered arachidonic acid (AA) biosynthesis and fatty acid composition in Sertoli and Leydig cells of diabetic rats, it was observed that insulin recovered AA biosynthesis, suggesting the participation of insulin also in the regulation of lipid metabolism. Based on the stimulatory effect of AA on testosterone secretion, the authors suggested that this might constitute another route by which insulin deficiency can impact male reproductive function (Hurtado de Catalfo & De Gomez Dumm 1998).

Furthermore, insulin resistance in men was observed to be associated with decreased testosterone production in response to hCG, further supporting a deregulation in Leydig cell function (Pitteloud *et al.* 2005a). In a follow-up study, the authors reported that the low testosterone levels observed were not only associated with insulin sensitivity but also with markers of mitochondrial function (Pitteloud *et al.* 2005b). Nonetheless, a recent study observed no relation between insulin resistance and male reproductive function (Verit *et al.* 2014). These contradictory results might be related to the different patients' selection criteria and number.

Besides the already mentioned effect of insulin on AA synthesis recovery, few are the studies regarding the effects of insulin replacement on Sertoli cell function of diabetics or in diabetic conditions. In 1983, Hutson described that ABP and FSH levels were altered in diabetic rats but can be recovered with insulin treatment, indirectly indicating a deregulation of Sertoli cell stimulation and function (Hutson *et al.* 1983).

More recently, testicular insulin levels were observed to be decreased in diabetic rats (Gomez *et al.* 2009), evidencing that testicular insulin deregulation might be behind the described metabolic and spermatogenic alterations in diabetic individuals. Aiming to clarify whether the reproductive problems associated with diabetes were due to a deficiency in systemic or testicular insulin (or both), Schoeller and coworkers performed an elegant study using as a model the Akita mouse, that presented altered fertility (Schoeller *et al.* 2012a). This mouse has a mutation in *Ins2* gene that results in a nonfunctional insulin expression in the pancreas and testis. Exogenous insulin treatment was observed to rescue the fertility impairment of these mice. The authors proposed that, due to the impermeability of the blood–testis–barrier to insulin, the observed defects in Akita mice fertility are mainly due to systemic alterations, highlighting the importance of pancreatic insulin in the regulation of the HPG. Furthermore, the authors described that Sertoli cells can produce insulin, suggesting a role of these cells in the regulation of testicular insulin signaling (Schoeller *et al.* 2012a). Nonetheless, this study did not disclose the exact role of testicular insulin and further studies are needed to clarify this point.

Post testicular level

Epididymis and sperm The epididymis is an essential organ of the male reproductive system, assuring sperm maturation. Therefore, any potential alterations in this organ, such as those occurring during diabetes (Soudamani *et al.* 2005, Singh *et al.* 2009) will most likely influence sperm function.

Insulin replacement was observed to have beneficial effects on some features of diabetic epididymis, partially recovering absolute weight, atrophic changes in the epithelium and the lumen diameter, thereby highlighting the importance of insulin in the maintenance of epididymal structure and function (Soudamani *et al.* 2005). Some years later, insulin was reported to also prevent, either partially or completely, the reduction in testosterone and ABP levels, secretory activity (carnitine, salicylic acid and glycerylphosphoryl coline) and concentrating capacity observed in the epididymis of diabetic rats, ultimately suggesting the involvement of insulin in sperm maturation too (Singh *et al.* 2009).

Regarding direct insulin effects on diabetic sperm, restoration of sperm motility and prevention of sperm count decrease was observed in several studies (Singh *et al.* 2009, Schoeller *et al.* 2012a).

Ejaculatory and erectile function

As pointed out, alterations at post-testicular level are also more common in diabetic individuals, as is the case of erectile dysfunction. Although some studies have reported beneficial effects of insulin treatment on some aspects of sexual function such as copulatory behavior and ejaculation (Steger & Kienast 1990, Yonezawa *et al.* 2009), few are the reports regarding the effects of insulin on erectile dysfunction (ED). Yet, this process also seems to be prone to the action of insulin. In fact, Yamanaka and coworkers reported that insulin treatment recovered the decrease in mean intracavernous pressure observed in diabetic rats and decreased the apoptotic index in penile crura (Yamanaka *et al.* 2003). The authors suggested that the insulin-promoted amelioration of the ED in diabetic rats might be mediated by the observed induced decrease in apoptotic index in the penile crura (Yamanaka *et al.* 2003) and similar results were reported by other authors (Wang *et al.* 2014, Choi *et al.* 2015). Furthermore, the observation that men with ED have a high incidence of insulin resistance and that this was positively correlated with increasing severity of ED, provides further evidence for a role of insulin in this process (Bansal *et al.* 2005).

In a subsequent study, insulin therapy, when given at an early stage of the disease, was found to completely prevent the alterations observed in the ejaculatory function of diabetic rats, such as spontaneous seminal emission, amount of seminal vesicle fluid or amount of ejaculated seminal material. The authors suggested that insulin is likely to have a role on the processes behind seminal emission and/or excretion in rats (Yonezawa *et al.* 2009).

Overall, acting at all the levels of the male reproductive system and reverting/attenuating the reproductive alterations

found on diabetic individuals, insulin has an unquestionable role on male fertility. In fact, insulin's positive effects have been reported at the hormonal level, in the testicular cells function (germ and somatic cells), in testicular metabolism, sperm function and also on ejaculatory capacity of diabetic individuals (Table 1). However, it is still to be determined how the oscillations in insulin levels or even the hypoglycemic episodes, which commonly occur during therapy, will impact the male reproductive function.

Biguanides

Biguanides are a well-established family of compounds used in the treatment of type 2 diabetes from which, the most used and studied one is metformin.

Metformin has been clinically prescribed for about 50 years, being the first-line drug of choice for the management of type 2 diabetes. This drug reduces hepatic glucose production, stimulates glucose uptake and increase insulin sensitivity in peripheral tissues, without causing hypoglycemia (Bailey & Turner 1996, Pirwany *et al.* 1999, Lochhead *et al.* 2000, Zhou *et al.* 2001, El-Atat *et al.* 2004). Metformin's mechanism of action, nevertheless, only recently began to be understood. It has been suggested that metformin action is achieved mainly through the inhibition of mitochondrial electron transport chain complex I, which leads to an increase of AMP:ATP ratio, that will then trigger AMPK, switching off the anabolic process. However, an action independent of AMPK has also been suggested with the direct metformin regulation of the gluconeogenic flux or mammalian target of rapamycin (mTOR) complex 1 inhibition. In essence, metformin can attenuate the two main pathways essential for mTOR complex 1 activation by (1) decreasing RHEB-mediated signals such as growth factors (IGF-1, insulin), cellular energy status (AMPK), ROS status and ER stress (ATM, REDD1) and (2) decrease amino acid-mediated PAT1/regulator-RAG signaling (Melnik & Schmitz 2014).

Although the main target of metformin is the liver, it can also act on other tissues/organs, including the reproductive ones. However, the effects of this drug on male reproductive function and fertility remain largely unexplored.

In fact, metformin treatment in diabetic rats has shown to increase body weight (Song *et al.* 2015), decrease fasting blood glucose (Adaramoye & Lawal 2014, Song *et al.* 2015) and significantly decrease diabetes-induced biochemical alterations mediated by oxidative stress, including sperm lipid peroxidation and altered antioxidant levels (Attia *et al.* 2009). Furthermore, this drug has been reported to restore sperm quality by promoting an increase in sperm count, motility and normal morphology, and overall, reducing sperm genomic instability caused by DNA fragmentation in diabetic rats (Attia *et al.* 2009). Moreover, when compared to pioglitazone (thiazolidinediones) and

sitagliptin (DPP-4 inhibitors/gliptins), metformin has shown to induce the smallest histological modifications on the structure of the testis, epididymis and seminal vesicles as well as restore their weights and promote an increase in testosterone levels (Ayuob *et al.* 2015). In both these latter findings, the values obtained were similar to the ones found in normal, non-diabetic rats (Ayuob *et al.* 2015).

Indeed, others have also reported an increase in serum testosterone levels with metformin treatment (Song *et al.* 2015) and an amelioration of the diabetes-induced testicular pathological changes (Kianifard *et al.* 2011, Song *et al.* 2015). Furthermore, while an increase in serum IGF-1 levels and testicular IGF-1 protein and mRNA expression were detected in metformin-treated rats when compared to the untreated diabetic ones, a decrease in growth hormone (GH) levels and testicular GH protein and mRNA expression were reported (Song *et al.* 2015). As the GH/IGF-1 axis has been reported to interact with the HPG axis and therefore regulate reproductive function, the observed alterations might explain the improvement of reproductive function following metformin treatment.

However, others have failed to detect any reproductive amelioration with this therapeutic approach. While no effects on absolute and relative testis weights (Adaramoye *et al.* 2012, Adaramoye & Lawal 2014) as well as on sperm live/dead ratio (Adaramoye *et al.* 2012) were reported in metformin-treated rats when compared to the untreated counterpart, a marked decrease in epididymal sperm count, motility and increased testicular lipid peroxidation was observed in this experimental group. Furthermore, these metformin-treated rats also presented altered testicular superoxide dismutase, catalase and glutathione activities and severe histological features including seminiferous tubules degeneration, spermatocytes defoliation and necrosis (Adaramoye *et al.* 2012). Nevertheless, it should be noted that the authors administered metformin to healthy adult rats with apparent normal glucose levels (Adaramoye *et al.* 2012), which though important to acknowledge metformin's toxicity, it is not quite relevant in a diabetes scenario. Later on, the same group was detected with an increase in sperm motility with metformin treatment to values even similar to the non-diabetic controls; however, no improvement was detected in sperm count or in total sperm abnormalities or sperm live/dead ratio even when compared to the untreated diabetic control group (Adaramoye & Lawal 2014). Indeed, metformin's action was so poor that also protein and sialic acid contents, glutathione-S-transferase and glutathione levels as well as antioxidant enzymes activities and lipid peroxidation were found unaltered in the testes of these treated animals when compared to the untreated diabetic group and were all altered in relation to the non-diabetic controls. The levels of testosterone, LH, FSH and prolactin were also reported to be similar to the

untreated diabetic males and decreased in comparison to the non-diabetic animals, prolactin being the only exception with no changes detected in the latter case (Adaramoye & Lawal 2014). However, the authors used alloxan to induce diabetes and the outcomes observed suggest metformin's limitation in the management of type 1 diabetes.

Besides type 1 and type 2 diabetes, gestational diabetes can also affect both the mother and the youngster and many reports have pointed toward a positive effect of metformin treatment during pregnancy. Yet, metformin has been shown to cross the placenta (Vanky *et al.* 2005, Kovo *et al.* 2008) possibly directly affecting embryonic and fetal development at concentrations approaching the ones found in maternal venous blood (Charles *et al.* 2006). In fact, studies of metformin exposure *in utero* have shown some testicular alterations such as diminished Sertoli cell number and reduced seminiferous tubules size in the male mice progeny (Bertoldo *et al.* 2014). Despite these, the effects of metformin administration during the development and function of the fetal testis has remained largely unexplored.

Using both human and murine organotypic testes cultures *in vitro*, Tartarin and coworkers have observed an increase in intratesticular lactate secretion upon exposure to 500 μ M metformin and higher, and reduction in both testosterone production and mRNA expression of factors involved in steroid production (Tartarin *et al.* 2012). Interestingly, cultured human fetal testes were found to be more vulnerable to metformin than their mouse counterparts as a concentration of 50 μ M, usually measured in patients' blood during therapeutic treatment, was enough to diminish testosterone secretion, while in mouse 500 μ M was required to promote the same effect (Tartarin *et al.* 2012). Such difference in sensitivity suggests caution when extrapolating the results, highlighting the need of rigorous comparisons among species. Furthermore, *in vivo* administration of metformin to pregnant mice at 300 mg/kg/day, a concentration similar to the one found to obtain a therapeutic effect in diabetic rodents (50 μ M), was shown to decrease testicular volume without increasing cell death in both fetal and neonatal periods and, in sharp contrast with what was detected *in vitro*, metformin failed to affect intratesticular lactate secretion. Testicular testosterone was found to be reduced but merely on male fetuses, which are in accordance with the reduced number of Leydig cells per testis only detected in the same period. Conversely, Sertoli cell number was found to be decreased in both fetuses and newborns (Tartarin *et al.* 2012). Overall, the combined *in vitro* and *in vivo* approaches to better evaluate the consequences of exposure to metformin highlight the potential harmful effect of such antidiabetic agent on testicular development. It should be noted, however, that some discrepancies, namely in lactate and testosterone secretion among *in vivo* and *in vitro*

approaches were observed, suggesting that one should be careful when analyzing the *in vitro* results and inferring to an *in vivo* situation or vice-versa. Although these results indicate that during pregnancy metformin should be used with caution, as it may result in gonadal developmental abnormalities, and further studies will be needed to clarify what will be the role of metformin in the offspring of (diabetic) mothers treated with this drug.

Sertoli cells, which are crucial for spermatogenesis, exert a tight metabolic control over such highly dynamic process. Recently, it has been reported that 50 μ M metformin induces a decrease in mRNA and protein levels of phosphofructokinase 1 (PFK 1), monocarboxylate transporter 4 (MCT4) and glucose transporters GLUT1 and GLUT3 in rat Sertoli cells cultured in high glucose conditions, but it has no effects on lactate dehydrogenase (LDH) mRNA or protein levels. However, an improved glycolytic flux was suggested as LDH activity and both alanine and lactate production were increased in metformin-treated Sertoli cells (Alves *et al.* 2014). Considering that glucose taken up by Sertoli cells is then converted into lactate via LDH and that such metabolite is necessary for energy production in the developing germ cells, regulating spermatocyte survival and metabolic activity, metformin has been suggested as a worthy drug for the management of male individuals diagnosed with type 2 diabetes (Alves *et al.* 2014). In support of such evidence is the inhibition of germ cell apoptosis by lactate in the human testis (Erkkila *et al.* 2002), leading the authors to propose that the treatment with a pharmacological dose of metformin may increase the male reproductive potential of diabetic patients via inhibition of germ cell apoptosis (Alves *et al.* 2014).

As previously mentioned, diabetic men commonly experience ED. However, the effects of metformin administration in this diabetes-mediated dysfunction have been deeply overlooked. Indeed, the majority of the existing literature has only addressed the effects of metformin on a non-diabetic ED context, and even so, the number of reports available is very limited. One of these is a prospective, randomized, controlled and double-blind placebo study where metformin was shown to improve erectile function and reduce insulin resistance in non-diabetic patients with ED and poor response to the phosphodiesterase-5 inhibitor sildenafil (Rey-Valzacchi *et al.* 2012). However, though considered mild and not significant enough to discontinue the treatment, patients treated with this drug presented more adverse effects than those receiving the placebo, mainly gastrointestinal effects (Rey-Valzacchi *et al.* 2012). Furthermore, Kim and coworkers have also reported that metformin is capable of restoring nitric oxide synthase protein expression in the penile tissue of rats fed with a high-fat diet (Kim *et al.* 2006) but whether such treatment could be beneficial to reestablish erectile function in an ED model was not known until 2013. At that time, using angiotensin II to promote hypertension

(which is considered a major risk factor for ED), Labazi and coworkers have shown that in angiotensin-treated rats, metformin increased eNOS phosphorylation and improved erectile function by re-establishing normal cavernosa smooth muscle tone (Labazi *et al.* 2013).

In summary, although limited, the available literature suggests that metformin might exert positive effects on the male reproductive function of diabetic individuals as other have also highlighted (Ferreira *et al.* 2015). Yet, its efficiency does not reach the observations made in non-diabetic controls (Kianifard *et al.* 2011, Ayuob *et al.* 2015, Song *et al.* 2015) (Table 1). Furthermore, when used in non-diabetic or in other than type 2 diabetes conditions, metformin might cause undesirable effects at the reproductive level, stressing the need of further studies to disclose metformin's pros and cons at this level. This should be performed mainly in humans, as a species-specific sensitivity seems to occur.

Sulphonylureas and meglitinides

Sulphonylureas (SUs) are a widely prescribed class of oral antidiabetic drugs for the treatment of type 2 diabetes mellitus (Ashcroft 1996). These compounds, introduced in 1942, stimulate insulin secretion from β -pancreatic cells, thus having a hypoglycemic ability (Janbon *et al.* 1942).

The first generation of sulphonylureas started with the introduction of tolbutamide followed by chlorpropamide, acetohexamide and tolazamide (Seltzer 1980). Later, a second generation was developed, which at that time included the compounds gliclazide, glyburide (glibenclamide), glimepiride and glipizide (Tommasini 1975, Kar & Holt 2008, Basit *et al.* 2012). Despite all acting similarly, they differ in the intrinsic antidiabetic activity, onset, period of action and elimination, and affinity for SU receptor (Jackson & Bressler 1981, Lebovitz 2001).

Although SUs have been the mainstay of pharmacotherapy for treating type 2 diabetes mellitus, some concern has been raised regarding their safety, as they contribute to increased risk of hypoglycemia and weight gain (Thule & Umpierrez 2014).

Through the inhibition of the ATP-dependent potassium channels (K_{ATP}), SUs stimulate insulin secretion in β -pancreatic cells, independently of blood glucose levels, and are referred as insulin secretagogues (Sturgess *et al.* 1985, Trube *et al.* 1986). Under normal conditions, these channels are open, allowing the efflux of potassium and polarization of the cell membrane (Burke *et al.* 2008). When sulphonylureas bind to these receptors the K^+ efflux is disrupted, which in turn leads to Ca^{2+} influx, resulting in cell depolarization and ultimately release of insulin-containing granules (Panten *et al.* 1996).

Besides β -pancreatic cells, these K_{ATP} channels have been observed in many other cells (Ashcroft & Gribble 1998),

including mouse spermatogenic cells and mature sperm, through the detection of mRNA levels and whole-cell patch-clamp/functional studies (Munoz-Garay *et al.* 2001, Acevedo *et al.* 2006). In these studies, the authors could provide evidence for the contribution of these channels to the hyperpolarization event occurring during sperm capacitation, and further hypothesized that they might also have a role in other important processes like spermatogenesis, sperm hyperactivation and acrosome reaction (Munoz-Garay *et al.* 2001, Acevedo *et al.* 2006).

Moreover, additional results were obtained with the identification of these K_{ATP} channels in epididymal epithelial cells from different mammalian species. Although the role of these channels in the epididymis is not clear, the authors suggested that they might be involved in several processes occurring along the epididymal epithelium, such as protein secretion and fluid-electrolyte transport, crucial for sperm maturation (Lybaert *et al.* 2008).

Since the presence of K_{ATP} channels on spermatogenic cells has been established, and knowing their role on sperm capacitation and its related events, it is important to analyze possible outcomes of sulphonylureas administration to (diabetic) male individuals.

Despite the persistent use of tolbutamide since its initial administration in 1956 (Scott & Poffenbarger 1979), few are the studies that report an effect on sperm and general spermatogenic activity. One of these studies is the already mentioned work by Acevedo and coworkers (Acevedo *et al.* 2006) that reported blocked hyperpolarization and a dose-dependent decrease in the percentage of acrosome-reacted mouse spermatozoa after exposure to this sulphonylurea in capacitating media. In contrast, no effects on sperm undergoing acrosome reaction were observed when the drug was tested after capacitation. These results indicate that tolbutamide prevents capacitation (Acevedo *et al.* 2006), highlighting the potential of this compound to interfere with the fertilizing capacity of male gametes. Nonetheless, the role of this drug in a diabetic context is still to be determined.

One of the first reports regarding the 'second generation' of SUs was on the sulphonylureas glibenclamide and gliclazide. This study has shown their spermicidal potential *in vitro* by describing a total loss of human sperm viability and motility, possibly due to an elevation of intrasperm calcium concentration ($[Ca^{2+}]_i$), that the authors proved to be resulting from the inhibition of the K_{ATP} channels (Kumar *et al.* 2008). In a different study, it was proved that glibenclamide exerted protective effects on diabetic rats' fertility (Rabbani *et al.* 2010b). Upon induction of a diabetic state through administration of nicotinamide-streptozotocin, the overall fertility parameters were affected showing an increase in shape abnormality and a reduction in sperm count as well as testis weight. However, after treatment with glibenclamide, there

was a decrease in shape abnormality, although no changes in sperm count were observed (Rabbani *et al.* 2010b). Similarly, the SU glimepiride was observed to have the same protective effect (Rabbani *et al.* 2009). The diabetic rats showed increased sperm morphology abnormalities and decreased sperm count and testis weight. Again, after treatment with the sulfonylurea, sperm morphological abnormalities decreased and, in this case, the authors observed an increase in sperm count also (Rabbani *et al.* 2009). Both studies suggested a protective role of glibenclamide and glimepiride at sperm level in diabetic conditions. Furthermore, as the authors observed a decrease in oxidative stress-related parameters after glibenclamide and glimepiride treatment, they suggest that the antioxidant properties of these drugs might reduce the oxidative stress-associated complications, including fertility problems (Rabbani *et al.* 2009, 2010b). However, different results were reported by Adaramoye and coworkers that, after glibenclamide treatment in healthy rats, observed a reduction in sperm motility and count while in the testis, lipid peroxidation was increased and the activities of antioxidant enzymes were decreased (Adaramoye *et al.* 2012).

Finally, gliclazide was also found to promote a significant increase in testicular 3 β -hydroxysteroid dehydrogenase (HSD) and 17 β -HSD enzymes' activity levels and serum testosterone levels in diabetic rats when compared to the untreated ones (Nelli *et al.* 2013), suggesting a positive effect of these SUs on the steroidogenesis of diabetic males.

No justification or suggestion has been made regarding the discrepant outcomes of SUs on male fertility. However, when analyzing the reported results, we can ascertain that when used *in vitro*, SUs have detrimental effects on capacitation and viability, both in human and mouse sperm cells (Acevedo *et al.* 2006, Kumar *et al.* 2008). On the other hand, when studies were performed in diabetic rats, the administration of SUs showed a positive effect at sperm and testicular level, reversing the adverse effects promoted by the disease (Rabbani *et al.* 2009, 2010b, Nelli *et al.* 2013) (Table 1). Nonetheless, a completely different scenario occurs when studies were performed in non-diabetic rats (Adaramoye *et al.* 2012).

Although when analyzing these studies there are several factors to consider, such as the type and dosages of SUs, the different species used or the specific parameters evaluated, it is reasonable to conjecture that while the beneficial effects of SUs *in vivo* might be a result of the overall action throughout spermatogenesis, resulting in sperm with higher quality, when *in vitro*, with the mature sperm cell as a final target, the panorama might be different. Furthermore, we should not disregard that while the antidiabetic properties of SUs might be effective in diabetic individuals and potentially translated in a better sperm quality, this might not be

the case in non-diabetic conditions, in which the drug might inclusively cause detrimental effects.

Furthermore, sulfonylureas therapy may have another downside. In fact, an increased risk of ED has been associated with these antidiabetic agents. Although the mechanisms behind this association are not fully unveiled, it has been suggested that the inhibition of K_{ATP} channels promoted by SUs might impair the vasodilation needed for the erection (Sehra *et al.* 2011). However, more studies are needed to fully disclose this issue.

Overall, although some controversy exists regarding the effects of SUs on the male reproductive function, these drugs are still often prescribed to type 2 diabetic patients, stressing the need of further studies to clarify the safety of this method in terms of male reproductive health.

In 1995, a new family of oral antidiabetic agents was introduced: the meglitinides analogues, also known as non-sulfonylurea insulintropic agents. This family is composed by repaglinide, nateglinide and mitiglinide (Malaisse 2003). Like sulfonylureas, meglitinides promote insulin secretion through K_{ATP} channels inhibition, increasing intracellular calcium concentration (Malaisse 1995, Landgraf 2000), and bind to the same receptor, the sulfonylurea receptor (SUR) (Dabrowski *et al.* 2001). However, these analogues are proposed to be superior to SUs, as they are not frequently linked to hypoglycemia occurrence (Malaisse 1995, Landgraf 2000).

Nonetheless, these compounds also seem to affect the sperm. In one of the few studies performed, besides sulfonylureas, the authors also used repaglinide. Similar to SUs, repaglinide produced a time- and dose-dependent decrease in human sperm viability resulting, ultimately, in the complete loss of viable male gametes. This outcome would also propose repaglinide as a spermicidal agent (Kumar *et al.* 2008). Nonetheless, the scarce information in terms of safety highlights the need of further studies.

Thiazolinediones/glitazones

Thiazolinediones (TZDs) or glitazones are another class of medication often used in the treatment of type 2 diabetes. These insulin sensitizer agents improve glycemic control, without causing hypoglycemia (ADA 2016d) and consist of pioglitazone, rosiglitazone and troglitazone.

Although troglitazone was the first to be approved for use back in 1997, it was quickly removed from the market due to the reported high frequency of liver injury (Gitlin *et al.* 1998, Neuschwander-Tetri *et al.* 1998, Herrine & Choudhary 1999, ADA 2016d).

Pioglitazone and rosiglitazone were approved in 1999, and they can be either used as a monotherapy or in combination with metformin, sulfonylureas or insulin. Contrarily to troglitazone, these have been rarely linked to acute liver injury. However, the increased risk of cardiac-associated events led the

European Medicines Agency to remove rosiglitazone from the European market. Nevertheless, it remains available in the US, although its use has been restricted. On the other hand, although reports suggested that the extended use of pioglitazone may significantly elevate the risk of bladder cancer, the evidence was considered insufficient to warrant its removal from the market by the health regulation organisms and in general, pioglitazone presents a more favorable safety profile, therefore being the most extensively used TZD (El-Kaissi & Sherbeeni 2011, ADA 2016d).

The antidiabetic effect of TZDs is accomplished by the activation of peroxisome proliferator-activated receptor in their gamma isoform (PPAR γ) (Lehmann *et al.* 1995). PPARs are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes (Berger & Moller 2002) and therefore participate in a wide range of processes, such as lipid homeostasis (Evans *et al.* 2004), angiogenesis, prostaglandin production (Desvergne & Wahli 1999), steroidogenesis (Gasic *et al.* 1998), inflammation, apoptosis, cellular proliferation and oxidative stress responses (Staels & Fruchart 2005).

As PPAR γ synthetic agonists, TZDs influence the transcription of several genes that participate in glucose and lipid metabolism and general energy homeostasis (Hauner 2002). In this way, TZDs reduce insulin resistance not only in adipose tissue, where the PPAR γ is mainly expressed, but also in the liver and muscle, probably through endocrine signals from the adipocytes (Hauner 2002).

Notably, the presence of PPAR γ was described in human sperm (Aquila *et al.* 2006) and testicular cells, including somatic (Braissant *et al.* 1996, Bhattacharya *et al.* 2005, Mansour *et al.* 2009, Thomas *et al.* 2011), and germ cells (Ibabe *et al.* 2005, Thomas *et al.* 2011) suggesting a potential role of TZDs on male fertility. Since diabetes is rising and leads to several health complications, including reproductive problems, it is therefore important to clarify the safety of these antidiabetic drugs at this level. Yet, in this review, we will not discuss troglitazone once it was on the market for a short period.

Rosiglitazone treatment, at a therapeutic dose, showed beneficial effects at the testicular level by reversing the detachment and disorganization of germ cells, a feature found in the seminiferous tubules of diabetic animals (Mansour *et al.* 2009). The authors suggested that these effects were probably due to an amelioration of the oxidative stress level, thought to be behind the testicular alterations observed in diabetic rats (Mansour *et al.* 2009).

Furthermore, although an inhibitory steroidogenic action by TZDs has been reported (Furnsinn *et al.* 2002, Vierhapper *et al.* 2003, Kempna *et al.* 2007), studies regarding TZDs effects in diabetic individuals' testosterone levels are scarce.

In non-diabetic men, rosiglitazone was observed to decrease serum testosterone and their production ratio (Vierhapper *et al.* 2003), while in rodents, no changes were reported in plasma testosterone levels (Couto *et al.* 2010), neither in the fetuses' testes after gestational exposure (Boberg *et al.* 2008). On the other hand, studies with diabetic animal models, reported a decrease in serum testosterone levels in diabetic fatty rats, as expected, and the treatment of these animals with rosiglitazone kept testosterone levels low, showing that rosiglitazone neither restored nor reduced serum testosterone levels (Mansour *et al.* 2009). Moreover, rosiglitazone treatment also did not alter the concentrations of the gonadotropins LH and FSH and serum estradiol, demonstrating no impact on the sex hormones of diabetic rats (Mansour *et al.* 2009).

There are also some studies that focused on the impact of rosiglitazone on testicular cells, however, not in diabetic conditions. To verify the ability of rosiglitazone to directly affect testosterone production by Leydig cells, Couto and coworkers performed an *ex vivo* assay using isolated Leydig cells from the testes of rosiglitazone-treated rats. Although the testosterone levels were not altered in the plasma of these animals, rosiglitazone modified the steroidogenic response of these cells resulting in a decrease in basal and induced testosterone production (Couto *et al.* 2010). Furthermore, ultrastructural analysis revealed alterations in the mitochondrial morphology that could have led to a mitochondrial dysfunction in Leydig cells, compromising steroids biosynthesis (important steps of which occur in mitochondria) and suggesting that the lower levels of testosterone produced by these cells could be due to mitochondrial damage induced by rosiglitazone (Couto *et al.* 2010). Nonetheless, it is still to be determined how rosiglitazone will act in diabetic conditions. In fact, though these studies are important as they provide information about the drug mechanism of action on testicular cells, they are irrelevant in terms of safety for diabetic individuals' reproductive function. Furthermore, the use of TZDs in non-diabetic settings, in which the insulin-sensitizing effects have no logical point, might blur the obtained results, thus avoiding the full understanding of the effects of these drugs on diabetic males' reproductive health.

Although there are no reports about the effect of rosiglitazone in sperm parameters of diabetic individuals, it was demonstrated that incubation of sperm from normozoospermic patients with rosiglitazone increased sperm motility and induced capacitation and acrosome reaction (Aquila *et al.* 2006). The authors also showed that rosiglitazone interfered with sperm glucose and lipid metabolism and, although not evaluated, this might constitute a way by which this drug can exert positive effects during diabetes due to an insulin sensitization action on sperm. Conversely, in a healthy rat model, rosiglitazone did not produce any effect on sperm

morphology and count (Rabbani *et al.* 2008), stressing the need of more evidence, not only to dissect the role of this drug on sperm function but also to get new insights about the possible consequences of rosiglitazone treatment in the sperm quality of diabetic patients.

In summary, although rosiglitazone was highly marketed for several years due to their antidiabetic properties, its effects on male reproductive system are not fully disclosed and more studies are needed to determine its safety having in regard the male reproductive function.

Currently, pioglitazone is the only TZD that remains to be prescribed, at least in Europe, and there are also some reports regarding its effect on male reproductive function. The studies of Gumieniczek and coworkers have focused on the effects of pioglitazone treatment (1 mg/kg/day/4 and 8 weeks) in the testicular tissue of alloxan-induced hyperglycemic rabbits (Gumieniczek *et al.* 2008, 2011). Based on the assumption that high levels of reactive oxygen/nitrogen species are present in the plasma of diabetic individuals and that this type of overproduction as well as inflammation is also evident at the testicular tissue level (Ceriello *et al.* 2001, Marfella *et al.* 2001, Shrilatha & Muralidhara 2007a), the authors investigated the effects of pioglitazone on these aspects. An amelioration in the activity of several plasma anti-oxidative enzymes, a reduction in testicular lipid and protein oxidation as well as myeloperoxidase levels, an enzyme released by inflammatory cells after infectious and/or inflammatory stimuli, and a normalization of aconitase enzyme activity, a sensitive target of reactive oxygen/nitrogen species and a readout of mitochondrial function, were observed in the testis of these hyperglycemic animals (Gumieniczek *et al.* 2008, 2011). It is therefore clear that pioglitazone treatment can modulate the oxidative stress associated with the diabetic condition exerting protective effects in hyperglycemic rabbit testis. However, in these studies, pioglitazone failed to affect blood glucose and insulin concentrations, probably because this diabetic rabbit model was not insulin resistant but insulin deficient. For this reason, in this case, the antioxidant effects of pioglitazone cannot be attributed primarily to its anti-hyperglycemic action, suggesting that this drug reduces free radicals' overproduction directly and indirectly through inhibition of inflammatory mechanisms.

The effects of pioglitazone on the male reproductive function were also studied in other animal models for diabetes as is the case of low-dose STZ-induced type 2 diabetic rats. These diabetic animals presented a reduced weight of reproductive organs (testis, epididymis and seminal vesicle), a disorganized germinal epithelium and an edematous thickened interstitial connective tissue, when compared to the non-diabetic controls. Pioglitazone treatment (20 mg/kg/day/6 weeks) increased the testis weight and caused a decrease in the epididymis weight. Furthermore,

pioglitazone-treated animals showed several histopathological changes in the testis, epididymis and seminal vesicles, that the authors hypothesized to be consequences of lipid peroxidation that remained high in these animals despite the treatment (Ayuob *et al.* 2015). Diabetes also negatively affected androgen, estrogen and their receptors (AR and ER respectively), known to have an essential role in male fertility; however, no improvements were observed after pioglitazone treatment that, on the contrary, was observed to cause a reduction in testicular ER α and ER β immunoexpression and to suppress ER β and AR immunoreactivity in epididymis and seminal vesicles (Ayuob *et al.* 2015). Based on these results, the authors warn for the use of pioglitazone on young diabetic males, as their reproductive capacity might be compromised. Although not discussed by the authors, it is possible that, the observed effects might also be due to the high dose (20 mg/kg/day/6 weeks) administered to these animals. In fact, it was shown that at a low dose (1 mg/kg/day) (Gumieniczek *et al.* 2008, 2011) even over a longer period (8 weeks) (Gumieniczek *et al.* 2008), pioglitazone exerts a clear protective effect in the modulation of oxidative stress associated with the hyperglycemic condition. Besides that, beyond the associated differences related to the species utilized, there is also a difference in the disease model since the pioglitazone-negative effects associated to a higher dose tested were reported in STZ-induced type 2 diabetic animals (Ayuob *et al.* 2015), while the positive effects found were reported in a type 1 hyperglycemic model (Gumieniczek *et al.* 2008, 2011).

The effects of pioglitazone on testicular cells are another important aspect. As Sertoli cells exert a crucial role in the spermatogenic process, the evaluation of their metabolism can be a good approach to identify possible effects of pioglitazone therapy. In human Sertoli cells cultured in high glucose conditions (that mimic diabetes), pioglitazone, at a pharmacological concentration (10 μ M), increased the glycolytic efficiency and stimulated the lactate production, which is essential to sustain and preserve spermatogenesis. At this concentration, pioglitazone did not alter the levels of mitochondrial protein complexes neither mitochondrial membrane potential. However, with the sub- and supra-pharmacological concentrations, some alterations were reported at the mitochondrial level, which once again strengthens the importance of dosage to obtain better treatment quality (Meneses *et al.* 2016).

Besides the testis, the effects of pioglitazone were also studied in sperm. In fact, based on the purported antioxidant properties of pioglitazone, Rabbani and coworkers investigated the effects of different concentrations of this drug on sperm morphology and count in healthy rats, after acute and chronic exposure (Rabbani *et al.* 2008). Although pioglitazone did not produce any variation in sperm count (chronic exposure at the highest dose tested –100 mg/kg/4 weeks),

a decrease in the number of defective sperm was noted, probably due to the antioxidant properties of pioglitazone (Rabbani *et al.* 2008). Nonetheless, this study had a major flaw in the experimental design that is the lack of a diabetic model. Therefore, in a following study, the authors used nicotinamide–streptozotocin-induced type 2 diabetic rats that presented the typical alterations in the antioxidant status and also in sperm parameters (Rabbani *et al.* 2010a). The authors reported that the higher dose of pioglitazone (10 mg/kg/4 weeks) reversed all the effects of the hyperglycemic status, presenting a decrease in blood glucose and lipid peroxidation, an increase in serum antioxidant GSH and GPx enzyme, as well as a decrease on sperm shape abnormalities accompanied by an increase in sperm count. This suggests that the beneficial effects of pioglitazone on sperm morphology of diabetic rats might be mediated by its antioxidant properties (Rabbani *et al.* 2010a), therefore being in accordance with the previous reports showing that the oxidative stress caused by hyperglycemia might be behind the altered seminal quality of diabetic individuals, compromising their fertility (Amaral & Ramalho-Santos 2014).

Finally, the diabetes-associated ED, which is mainly a vasculogenic problem caused by arterial insufficiency and corporal veno-occlusive dysfunction (CVOD), has been poorly addressed in an antidiabetic treatment context. Yet, diabetic (fa/fa) rats with penile corporal fibrosis, oxidative stress and CVOD presented improvements in these aspects by long-term, low-dose (0.6 mg/kg) pioglitazone treatment with no reduction in glycemia, suggesting a vasculoprotective role of this drug, independent of its antidiabetic action (Kovanecz *et al.* 2006).

We can conclude that besides the antidiabetic one, pioglitazone present other properties, as antioxidant and anti-inflammatory, that might mediate improvements in male reproductive function (Table 1). Yet, this seems to also depend on the drug dose administered or animal model used, which might in fact be the basis of the contradictory reports. Therefore, the use of pioglitazone should be restrained in young diabetic males while more consistent data are not gathered regarding this topic.

GLP1 and DPP4 inhibitors

Glucagon-like peptide (GLP1) is an incretin, a hormone produced by the small intestine cells following nutrient intake, whose levels are decreased in type 2 diabetic patients. This hormone induces the secretion and release of insulin and, in animal models, it was shown to promote an increase in the β cell mass (Hvidberg *et al.* 1994, Wajchenberg 2007). Furthermore, GLP1 decelerates gastric emptying causing a satiety feeling and diminishes postprandial glucagon production, thus contributing to a decline in blood glucose levels (El-Kaissi & Sherbeeni 2011). A role in lipid metabolism

regulation has also been reported, suggesting that this compound can contribute to weight loss (Dallinger-Thie & Nieuwdorp 2015). However, the half-life of this substance *in vivo* is of about two minutes due to its rapid metabolism by the dipeptidyl-peptidase-4 (DPP-4) enzyme (Gupta 2013).

Currently, the GLP-1 analogues available and approved by the Food and Drug Administration (FDA) for type 2 diabetes treatment are exenatide, dulaglutide, albiglutide and liraglutide that differ mainly in dosage and type of administration (Miller *et al.* 2014). Nonetheless, not much information exists regarding their effects on male reproductive function.

In the first report regarding this subject, researchers tested the effect of exendin-4 (endogenous bioavailable version of exenatide) on the male reproductive function of healthy mice (Ahangarpour *et al.* 2014). It was observed that the administration of 1 and 10 nmol/kg of exendin-4 increased epididymal sperm count and testis weight. Furthermore, both treatments led to a decrease in the LH and FSH levels that, in the lower dosage, was accompanied by an increase in testosterone levels (Ahangarpour *et al.* 2014), suggesting that exendin-4 interfered in the HPG regulation.

Posteriorly, Zhang and coworkers evaluated the treatment of high-fat-induced obese mice with exenatide on their fertility (Zhang *et al.* 2015). Exenatide was observed to reverse the negative effects of obesity on sperm function by increasing sperm motility and mitochondrial membrane potential while reducing DNA damage. Also, the treatment did not change the volume of the testis nor affected the testosterone levels. Furthermore, a reduction in the expression of pro-inflammatory cytokines in the testis, like TNF- α , MCP-1 and F4/80, was observed (Zhang *et al.* 2015).

The different effects of exenatide on testosterone levels and testis weight among studies may be due to the fact that in the first study, the authors used normal rats, with apparently no metabolic defects, while in the later, the authors used an obese diabetic animal model, in which insulin resistance, as well as metabolic deregulation is highly probable (Gomez-Hernandez *et al.* 2016). Therefore, in this last study, the beneficial effects might have been mediated by an improvement in insulin signaling and overall metabolic status. Moreover, the observed anti-inflammatory effects of this drug might have also played a role, as suggested by the authors.

In humans, there is a single case report in which it was observed that the treatment of a diabetic man with the GLP1 liraglutide led to interrupted sperm production, which was restored after 5 months without taking the substance. The authors proposed that this was due to a reduction of testosterone pulses induced by the infusion of that GLP1 analog (Fontoura *et al.* 2014).

Overall, from the data we presented here, it is clear that more studies are needed to disclose the exact role of

GLP-1 on male reproductive function, in particular that of diabetic individuals.

Another explored method to increase incretin hormones endogenous levels is by preventing their rapid degradation through the inhibition of the enzyme dipeptidyl peptidase 4 (DPP4). Sitagliptin, saxagliptin, linagliptin, vildagliptin and alogliptin are the DPP4 inhibitors currently in use, the first being the most studied one as it was the first to be approved for therapeutic purposes (Stein *et al.* 2013). Unfortunately, their effects on male reproductive function remain largely unexplored.

The first evidence was from a case study of a diabetic man treated with sitagliptin (50 mg/daily), that showed a progressive decrease in semen volume as well as in sperm concentration and motility (Hibi *et al.* 2011). Furthermore, the patient presented low levels of free testosterone. For that reason, the treatment was discontinued and the following semen analysis revealed a recovery of semen parameters (Hibi *et al.* 2011). This case report suggested that DPP-4 inhibitors may have a negative effect on diabetic male's fertility. However, since then, few studies were conducted to clarify this subject.

In a more recent work, the treatment of STZ-induced diabetic rats with sitagliptin (30 mg/kg/day) slightly decreased the pathological changes caused by diabetes in terms of structure and weight of the testis, epididymis and seminal vesicle (Ayuob *et al.* 2015). However, the treatment was not able to achieve a complete reversion of the diabetic phenotype. Furthermore, despite the fact that the testis of sitagliptin-treated rats presented the highest number of spermatocytes in different mitotic stages, it was observed that the treatment failed to recover testosterone levels. Additionally, rats under sitagliptin treatment presented decreased expression of estrogen and androgen receptors in the epididymis and in seminal vesicles (Ayuob *et al.* 2015). For these reasons, the authors suggested that this treatment should be avoided in young male diabetics.

In summary, although the studies on this topic are scarce, the available information points to a rather adverse effect of these antidiabetic agents on male fertility (Table 1), warning for their use in diabetic males, at least during their reproductive age, until more information is available regarding their safety at this level.

Other antidiabetic drugs

In this subsection, we will discuss reports regarding the effects of other antidiabetic drugs, less used and studied, such as α -glucosidase inhibitors, bile acid sequestrants, sodium-glucose co-transporter 2 (SGLT2) inhibitors and amylin analogues.

The α -glucosidase inhibitors act by inhibiting the intestinal α -glucosidase, that will result in delayed carbohydrate absorption and lowering of blood glucose levels. They do not directly target insulin production but instead promote GLP1 hormone secretion increase (Stein *et al.* 2013).

The two FDA-approved α -glucosidase inhibitors for type 2 diabetes treatment are acarbose and miglitol (Stein *et al.* 2013) and their effect on male fertility is still to be addressed. In fact, to our knowledge, there was only one study that evaluated the effect of acarbose on the fertility of STZ-induced diabetic rats (Sadeghi *et al.* 2013). It was observed, that the reduction on the numbers of spermatogonial cells, spermatocytes and Sertoli cells in the testis was lower in the diabetic rats treated with acarbose than that detected on untreated diabetic rats (Sadeghi *et al.* 2013), suggesting that this drug might have positive effects on reproductive aspects of diabetic individuals. Nonetheless, further research on this topic should be of utmost importance.

Regarding bile acid sequestrants, although they were originally prescribed to treat hyperlipidemia, they were also found to reduce blood glucose levels (Garg & Grundy 1994). The responsible mechanism for this action is not completely understood, but the activation of farnesoid X receptor or the stimulation of incretin hormones secretion might be involved (Stein *et al.* 2013). Currently, there is only one bile acid sequestrant approved for diabetes treatment, colestevam, normally prescribed in combination with other antidiabetic drugs (Stein *et al.* 2013).

We could find only one study regarding the effects of colestevam on male fertility. This study, conducted in Sprague–Dawley rats, reported no differences on sperm concentration and motility neither in matting and litter size, between the controls and the treated groups (Marquis *et al.* 2004). Based on these results, colestevam does not seem to be detrimental for male reproductive function, but this does not exclude the need of further research to clarify this subject.

Besides all the mentioned antidiabetic drugs, there are also sodium-glucose co-transporter 2 (SGLT2) inhibitors, that lead to a reduction in blood glucose levels by increasing glucose urinary excretion (Cuypers *et al.* 2013, Ji *et al.* 2014). Importantly, the increase of glucose levels in the urine augments the incidence of urinary and genital tract infections in males (Cuypers *et al.* 2013, Ji *et al.* 2014); however, there are no other reports on the effects of this drug in the male reproductive system. On the other hand, there are also the amylin analogues, whose secretion is impaired in diabetic patients (Young 2005). Amylin is co-secreted with insulin and promotes the reduction of blood glucose levels, suppresses glucagon production and reduces

satiety (Hoogwerf *et al.* 2008). However, to the best of our knowledge, no studies exist regarding the effects of these compounds at the male reproductive level.

Concluding remarks

The impact of diabetes on male reproductive function is concerning mainly due to the alarming increase of the disease worldwide, which is translated into augmented chances of affecting males at the acme of their reproductive window thus contributing to the general decline in fertility rates.

Considering that the cornerstone for the disease management is the glycemic control, achieved through several therapeutic approaches, it is of utmost importance to understand how these treatments affect the male reproductive health, to guarantee that this is maintained during antidiabetic therapy. As this issue is not commonly addressed, we made a comprehensive analysis and a critical review of the available reported data.

While for insulin no doubts exist regarding its importance for male reproductive function, easily attested by the promoted recovery of the reproductive impairment in insulin-dependent diabetic individuals, and for other treatments, this connection is not straightforward. In fact, studies with other antidiabetic drugs originated controversial results that, in our point of view, might be related with marked differences in the study design. Specifically, one of the main constraints that we have found was the use of these drugs in non-diabetic conditions, which we believe might have influenced the obtained results. In fact, if the action of these drugs is directed to improve glycemic control and revert diabetic-associated alterations, and, unlike insulin, they do not normally exist in the body, it is logical to suggest that while studies performed in non-diabetic conditions might be useful to clarify some details regarding their mechanisms of action, the biological relevance in a disease scenario is questionable. In fact, we could detect that, in these conditions, even detrimental effects were reported, contrasting with the positive effects observed in diabetic conditions.

Nonetheless there are still questions to be answered, such as, what will be the influence of the insulin levels variation, that occur during therapy, as well as of the hypoglycemic episodes, a common consequence of several therapies, at the male reproductive level. Therefore, it is crucial to continue research on this topic.

Overall, this stresses the need to engage diabetic patients in informative actions regarding these topics, making them aware not only about the possible impact of diabetes on their reproductive health and fertility potential, as well as of the associated therapies, ultimately mobilizing them to participate in clinical trials. On the other hand, this will expand our knowledge on these topics with the ultimate goal of improving reproductive healthcare.

Declaration of interest

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

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