

# Chapter 2

## Biological Aspects of the Mature Boar Spermatozoon

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**Abstract** A practical consequence of the particular reproductive cycle of sows is that the functional features that distinguish boar spermatozoa cannot be extrapolated to other species, thus preventing an overall picture that explains mammalian sperm function from being assumed. Furthermore, the extraordinary complexity of the molecular mechanisms implied in the control and modulation of mature boar sperm functions makes it impossible to provide a complete description of these mechanisms in the limited space of this chapter. Taking this into account, this chapter centers on the description of three highly important specific aspects of boar sperm function. The first aspect is the mechanisms by which boar sperm cells manage their energy levels. The second aspect is the functional role of mitochondria as controllers of boar sperm function. The third aspect will address the existence of functional, separate subpopulations in boar ejaculates, and the hypothetical biological role of these subpopulations.

### 2.1 Introduction

The mammalian spermatozoon is a very specialized cell designed to accomplish an ultimate goal: the transmission of the paternal genome to the next generation. To achieve this goal, mammalian spermatozoa are finely designed, following the specific evolutionary reproductive strategy chosen by each species. This specificity of design implies that mature mammalian sperm are very complex cells, with several functional features that clearly distinguish them from other eukaryotic cells. Focusing on swine, boar spermatozoa are perfectly adapted to a female reproductive cycle based on oestrous phases of about 2–3 days, poly-ovulatory events, and a

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tendency of the sow toward monogamy. This differentiates boar sperm functionality from other species and thus, their sperm must accomplish particular steps in order to yield optimal “in vivo” fertility rates.

## 2.2 Mature Boar Sperm Energy Resource Management

Control mechanisms of boar sperm energy management play an essential role. This is due to the fact that practically all the reactions that maintain the functional status of the cell (control of tyrosine phosphorylation levels, maintenance of the glycosylation of membrane proteins, etc.), as well as those related to the maintenance and further control of molecular mechanisms controlling sperm resistance to environmental changes, need significant energy consumption. Thus, the optimal function of all these mechanisms will depend, to a great extent, on a correct functioning of control mechanisms modulating boar sperm energy management. Unfortunately, and despite vast amount of knowledge accumulated by many investigators in the past 20 years, there are several commonplaces regarding sperm energy metabolism, which, in fact, obstruct an optimal practical application of this knowledge. Thus, everybody knows that the mammalian spermatozoon is strictly a glycolytic cell. However, the adoption of this assertion can undoubtedly lead to the opinion that spermatozoa are almost exclusively glycolytic, therefore, they have practically no other modulator system to manage their energy levels (Mann 1975). On the other hand, if the spermatozoon is an exclusively glycolytic cell, what is the role of sperm mitochondria and the associated mitochondrial respiration? In this respect, it is noteworthy that many investigators indicate as an absolute fact that the energy obtained through the mitochondrial respiration is, under all conditions, absolutely necessary for the maintenance of sperm motility in all species (Nevo et al. 1970; Ford and Harrison 1985; Halangk et al. 1985; Folgero et al. 1993; Ruíz-Pesini et al. 1998), despite the fact that the same investigators maintain the absolute preeminence of glycolysis to obtain sperm energy, without realizing the contradiction in terms of energy that the simultaneous assumption of both principles implies. These contradictions highlight the complexity of the question, which has to be approached with an open mind. Only in this way can some valid and general conclusions with practical applicability on sperm conservation be attained.

Taking into account all these aspects, the study of boar sperm energy management can be structured around the following questions:

- First question: what energy sources can be utilized by mature boar sperm during their journey inside the female genital tract?
- Second question: what are the main metabolic pathways by which these energy sources are utilized?
- Third question: what are the main control mechanisms that coordinate energy management and changes in boar sperm function?

### ***2.2.1 Energy Sources of Mature Boar Sperm***

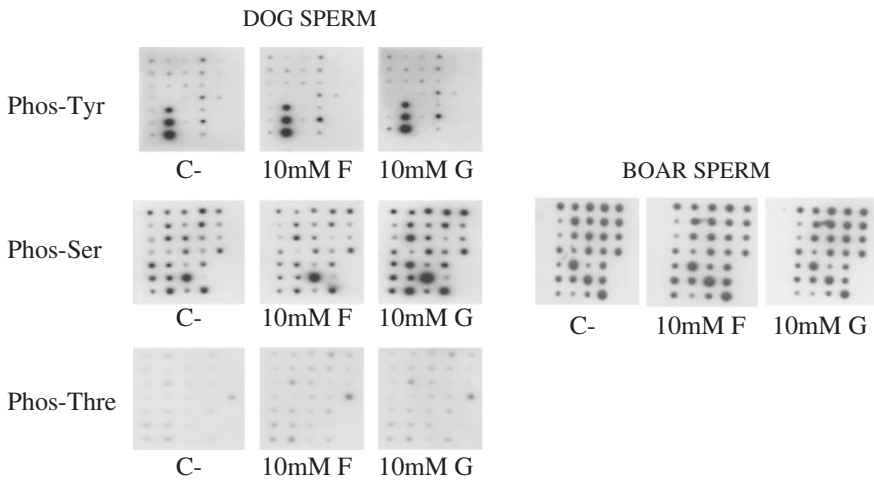
A general consensus exists among researchers that the main external energy sources of mature boar sperm are monosaccharides. In this way, it has been described that boar spermatozoa are able to metabolize a great variety of monosaccharides, from the ubiquitous glucose to other less common sugars, such as fructose, sorbitol, and mannose (Mann 1975; Jones and Connor 2000; Rigau et al. 2002; Marín et al. 2003; Medrano et al. 2006a). However, it is noteworthy that the fact that boar sperm is able to metabolize a specific sugar does not imply that this sugar is a usual energy substrate. In fact, the ability of boar sperm to utilize a specific monosaccharide is different depending on the utilized sugar. In this sense, it has been described that hexokinase activity, which phosphorylates monosaccharides thereby enabling their utilization through the cellular metabolic pathways, is more sensitive to glucose than to other monosaccharides, such as fructose, sorbitol, and mannose (Medrano et al. 2006a). This implies that glucose will be utilized faster than the other monosaccharides and, in this way, glucose will be a more suitable energy source than, for instance, fructose. However, there are other factors modulating the different availability of monosaccharides for boar sperm energy production. In fact, the first control step in monosaccharide utilization is not sugar phosphorylation, but monosaccharide uptake from the extracellular environment. This uptake is carried out through specific transmembrane transporters. To date, several hexose transporters have been described in boar spermatozoa. These are mainly of the GLUT family transporters. Boar spermatozoa present at least four separate GLUTs, namely GLUT-1, GLUT-2, GLUT-3, and GLUT-5 (Bucci et al. 2010). These transporters have separate affinities for specific monosaccharides, as well as presenting different hexose uptake rates. Thus, GLUT-3 is a highly specific glucose transporter, whereas GLUT-5 is completely fructose-dependent (Mueckler 1990). The presence of separate transporters with highly different affinity and uptake rate characteristics entails a complex and very sophisticated system to control hexose uptake. This is further complicated by the existence of a very specific distribution of each GLUT over the entire sperm surface. Thus, GLUT-3 is preferentially placed at the midpiece, whereas the preferential location of GLUT-5 is at the periacrosomal area, although researchers differ as to the utilized fixative technique (Medrano et al. 2006a; Sancho et al. 2007; Bucci et al. 2010). These specific locations indicate that a specific hexose, like glucose, can only be taken up by boar sperm through specific points of the cellular membrane, initiating thus its metabolism at strictly limited locations in the boar sperm cellular structure. We can only speculate about the biological significance of this strict hexose uptake location, although it seems to indicate the existence of directionality in hexose metabolism, by which a precise monosaccharide would be metabolized in a precise location inside boar sperm. This directionality, in turn, would indicate that hexose metabolism could follow specific and separate pathways depending on the precise uptake point. This would imply in turn that mammalian sperm regulate hexose utilization not only through the control of the enzymatic activities that modulate this

utilization, but also through the presence of specific metabolic pathways with a precise, spatial location inside the cell. These spatial mechanisms would allow for a better optimization of the utilization of energy sources, since they are metabolized in an almost instantaneous manner throughout the metabolic pathway, which could render maximal efficiency depending on the specific physiological status of the sperm.

Sugars are not the only extracellular substrate that boar sperm can utilize to obtain energy. Boar sperm can also utilize non-hexose compounds, such as lactate, pyruvate, glycerol, and citrate (Jones et al. 1992; Jones 1997; Medrano et al. 2006b). The role of these substances as energy sources of mature boar sperm is, however, not well understood. These compounds do not seem to be present in significant amounts, at least, several of them like glycerol, are either in seminal plasma or inside the female genital tract. Moreover, whereas some metabolites, such as glycerol, can be metabolized by glycolysis, others like lactate, pyruvate, and citrate will be directly metabolized through the mitochondrial Krebs cycle. This poses a problem, since the energy yielding rate of boar sperm mitochondria is very low, about two magnitude orders below that determined for other highly energy-producing mitochondria, such as those from pig hepatocytes (Balis et al. 1999; Ramió-Lluch et al. 2011). This implies that these substrates are, in fact, inefficient at providing energy, especially when compared to monosaccharides. This low efficiency seems to point another role of these substrates other than merely obtaining energy. In this regard, it is noteworthy that citrate is directed by boar sperm to the Krebs cycle, but the obtained citrate-derived metabolites are subsequently processed through the pyruvate carboxylase step, finally synthesizing lactate. This lactate is subsequently secreted to the extracellular medium and then reentered into the Krebs cycle through the lactate dehydrogenase step. At a first glance, this very complicated utilization mechanism is not easy to understand. Nevertheless, this mechanism will yield important amounts of the reductive agent  $\text{NAD}^+$ , which is of paramount importance to stabilize sperm function (Baker and Aitken 2004). In this manner, these substrates would be primarily utilized to obtain reductive potential, energy production being thus a less important function.

There is a final point of interest regarding the sources of boar sperm energy substrates. What is the origin of these sources? It seems clear that mature boar sperm, after ejaculation, can only obtain their energy substrates in two ways. The first way will be from compounds present in the seminal plasma. The second way will be from substrates already present inside the female genital tract before sperm entry. Regarding substrates from seminal plasma, it is noteworthy that the great volume of boar ejaculate allows spermatozoa to maintain contact for a time with substances contained in the seminal plasma. This is different from other species, such as bovine or human, in which the sum of a low volume ejaculate with a fast semen deposition inside a genital tract or a relatively great volume implies that the contact of sperm from these species with compounds of seminal plasma will be brief. Moreover, boar seminal plasma

has been described to contain measurable amounts of glucose, although there are great discrepancies regarding the real concentration of the sugar (Baronos 1971; Mann 1975). This is important if we remember that glucose is the most efficient sugar to produce energy for boar sperm (Medrano et al. 2006a). Other sugars, like fructose and inositol, are also present (Baronos 1971; Mann 1975), thus the question arises of what the exact role of these minority compounds is in boar ejaculates. One possibility is that minority energy substrates could play another role than just being an energy source. We have already explained how boar sperm metabolizes citrate and the primary role of this compound as source of reduction potential (Medrano et al. 2006b). A similar role could be played by other substrates, like lactate. Another role could be that of a direct functional modulator by acting on, for instance, tyrosine phosphorylation levels of specific intracellular proteins. This effect has been demonstrated for both glucose and fructose in dog, but not in boar sperm (Fernández-Novell et al. 2011 and Fig. 2.1). However, this lack of effect does not necessarily imply that a sugar-specific action on some specific intracellular proteins is not present in boar sperm, and further investigations are needed to elucidate the role of minority substrates found in both seminal plasma and the female genital tract.



**Fig. 2.1** Mini-array analysis of the tyrosine, serine, and threonine phosphorylation status of several proteins involved in the regulation of cell cycle and overall cell function in dog and boar spermatozoa after incubation with glucose or fructose. Dog and boar spermatozoa were incubated for 5 min in the absence (C-) or presence of either 10 mM fructose (10 mM F) or 10 mM glucose (10 mM G). The tyrosine- (Tyr-Phos), serine- (Ser-Phos), and threonine-phosphorylation (Thre-Phos) levels of each spot in the mini-arrays were then analysed. The figure shows a representative image for five separate experiments. Figure taken from Fernández-Novell et al. (2011)

### ***2.2.2 Main Metabolic Pathways for Energy Production in Mature Boar Spermatozoa***

As indicated above, the most important metabolic pathway by which mature boar sperm obtain energy is glycolysis. In a metabolomic study, it has been observed that approximately 95 % of the energy yielded by glucose in boar sperm originates through the glycolytic pathway, the mitochondrial respiration-produced energy being only about 5 % of the total glucose energy yield (Marín et al. 2003). At a first glance, this result can seem contradictory, since the glycolytic energy yield is much lower than that obtained through other metabolic pathways like mitochondrial respiration. Thus, glucose is not efficiently metabolized by boar sperm in these conditions. However, there is an aspect that could explain this apparent contradiction. As any biochemistry student knows, the mitochondrial respiration requires an aerobic environment to work in optimal conditions, whereas glycolysis is highly efficient in an anaerobic environment. The environment that mature boar sperm will find after ejaculation inside the female genital tract would be, generally speaking, mostly anaerobic. In these conditions, the mitochondrial respiration will not work at good rates, whereas glycolysis will. Taking into account this aspect, it is logical that the overall metabolic machinery of mature boar spermatozoa has been designed to maximize the generation of energy in anaerobic conditions, thus giving a prominent role to the glycolytic pathway. Obviously, all these results do not preclude the existence of a precise role for the energy obtained through the minority mitochondrial respiration route. Thus, it has been observed that the achievement of a feasible, progesterone-induced “in vitro” acrosome reaction is concomitant with a rapid and intense increase in the oxygen consumption rate, which indicates a rapid, transitory, and intense peak of the mitochondrial respiratory activity (Ramió-Lluch et al. 2011). This seems to indicate that this minority energy would be necessary for the achievement of acrosome reaction; meanwhile glycolysis-synthesized energy would be the main energy source for all other boar sperm necessities.

The assumption of a main glycolytic source for obtaining energy in boar sperm suggests that energy production is a very fast phenomenon, mainly controlled by the rate of exogenous substrates and subsequent phosphorylation. However, several data seem to indicate that this overview is more complex. Thus, it has been shown that boar sperm are able to accumulate energy resources in the form of glycogen in a limited way (Medrano et al. 2006a). This is similar to that observed in other species, such as dogs and horses (Ballester et al. 2000), although the role of an active glycogen metabolism seems to be different among species. Thus, glycogen metabolism in dog sperm is related to the maintenance of energy levels during “in vitro” capacitation through a complex mechanism involving glycogenesis and an indirect glycogen synthesis pathway (Albarracín et al. 2004). Boar sperm, instead, do not show signs for the existence of a functional gluconeogenic pathway from substrates like lactate or citrate (Marín et al. 2003). This implies that glycogen must be obtained directly from phosphorylated substrates that are

diverted at the start of the glycolytic pathway. We do not know what is the exact role of boar sperm glycogen. Taking into account its origin, this glycogen could be a supplementary source of energy in case of an urgent need for energy supply. Notwithstanding, more information is needed to elucidate this intriguing characteristic of energy metabolism in mature boar spermatozoa.

### ***2.2.3 Control Mechanisms Involved in the Coordination of Energy Management of Boar Sperm***

Information regarding the control mechanisms of energy management in boar spermatozoa is scarce. Recompilation of data from several authors indicate the existence of two main check points in the glycolytic pathway, which is the main energy source for boar sperm as indicated above. The first check point has been already described, since it is linked to the uptake and subsequent phosphorylation of exogenous substrates. Thus, the placement and activity of GLUT transporters and subsequent hexokinase activity will regulate this point. Taking into consideration the specific characteristics of both GLUTs and hexokinase activity, it seems evident that GLUTs perform the most relevant control role. This is due to the fact that overall boar sperm hexokinase activity is very fast and, moreover, reaches its maximal level at very low substrate concentrations and at micromolar orders (Fernández-Novell et al. 2004; Medrano et al. 2006a). This implies that practically all the substrates taken up by the cell are immediately phosphorylated and processed either toward glycolysis or glycogen synthesis (another possible pathway, the pentose phosphate cycle, was not detected in metabolomic studies, see Marín et al. 2003). In fact, the velocity of this step is such that boar sperm rarely achieve their theoretical stoichiometric ATP yield (Hammersted and Lardy 1983). In this way, a hexokinase-linked regulation of hexose metabolism is almost impossible. On the other hand, the presence of at least four separate GLUTs with different characteristics of uptake velocity and hexose specificity creates a more complex control system, in which the metabolism of a specific hexose will vary depending on the specific transporter that makes contact with this specific hexose. This is further complicated by the fact described above of the heterogeneous presence of each GLUT in specific sperm membrane positions. This will mean that, for instance, fructose will be taken up and subsequently metabolized at a much faster rate in the post-acrosomal region than in the acrosomal area, since the distribution of the GLUT-5, fructose-specific transporter is much more abundant in the post-acrosomal area (Sancho et al. 2007). This also seems to indicate the existence of metabolic zonation, in which separate areas of boar spermatozoa would have different rates of energy management, depending on the velocity and specificity of the relevant substrates in each separate sperm location.

Notwithstanding, there is another important check point to regulate glycolytic flux. This second check point involves modulation of the lactate dehydrogenase

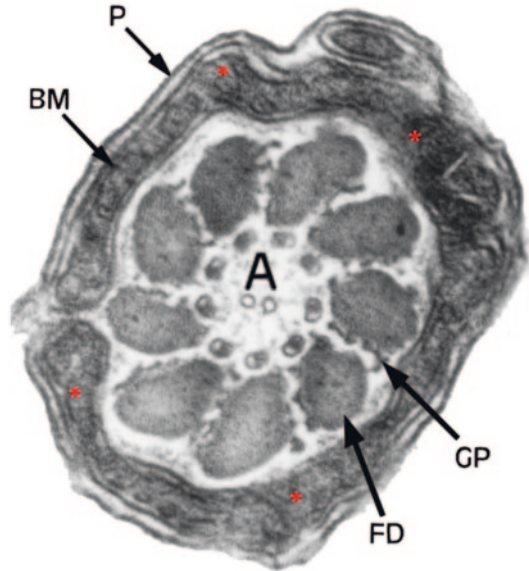
activity (LDH), which controls the final fate of the obtained lactate, directing it either to the Krebs cycle or its secretion to the extracellular medium. It is noteworthy that mammalian spermatozoa present a specific LDH isozyme, which is mainly located at the principal piece of the tail (Jones 1997; Medrano et al. 2006b). The regulation of this LDH is very complex, involving the migration of the LDH protein from an insoluble cellular fraction, presumably linked to the axoneme of the principal piece of tail, to a soluble cellular one, loosely linked to the inner tail structures (Medrano et al. 2006b). Thus, boar sperm LDH regulates its activity by changes of its position inside a specific cellular sector. This system of regulation seems to imply the existence of a regulation mechanism based on the spatial contact among all the constituents of the entire glycolytic pathway. This pathway would work at different rates depending on the specific positions of some of the enzymes implied in glycolysis. In this way, changes in the location inside a precise zone of the sperm structure of proteins like GLUT-3, GLUT-5 or LDH would automatically lead to changes in the rate of the glycolytic flux. This would be a precise mechanism able to modulate rapid and intense changes in the energy formation rhythm, thus allowing for a very fine modulation mechanism of boar sperm intracellular energy levels.

### **2.3 Roles of Mitochondria in the Control of the Overall Mature Boar Sperm Function**

As indicated in the previous section, the main energy source for mature boar sperm are ATPs obtained through the glycolytic pathway, with a minority role for mitochondria-originated energy through the mitochondrial respiration. As a result, an important question is raised: if boar sperm mitochondria do not seem to have a predominant role in obtaining energy, what is their main role? We can only speculate on this point, although several data can help us gain greater insight into this issue. The first data correspond to the observation of the boar sperm mitochondria ultrastructure (Fig. 2.2). Electron microscopic images of boar sperm mitochondria show an organelle with few and small inner membrane cristae. Instead, the inner mitochondrial space is mainly occupied by an amorphous and homogeneous matrix. This is very different to the classical image for mitochondria, which, like those from hepatocytes, show an inner structure crowded with prominent inner cristae (Fig. 2.2). Taking into account the most important steps of the electronic transport system and subsequent ATP synthesis are structurally linked to inner mitochondrial cristae (this information can be obtained from any of the excellent biochemistry books for students utilized worldwide), it is easy to assume that boar sperm mitochondria would not be very efficient as energy suppliers. In fact, as described above, the oxygen consumption rate of boar sperm, which is a direct measure of mitochondrial ability to generate energy, is about 2 magnitude orders lower than that measured in pig hepatocytes (Balis et al. 1999; Ramió-Lluch et al. 2011). This further reinforces

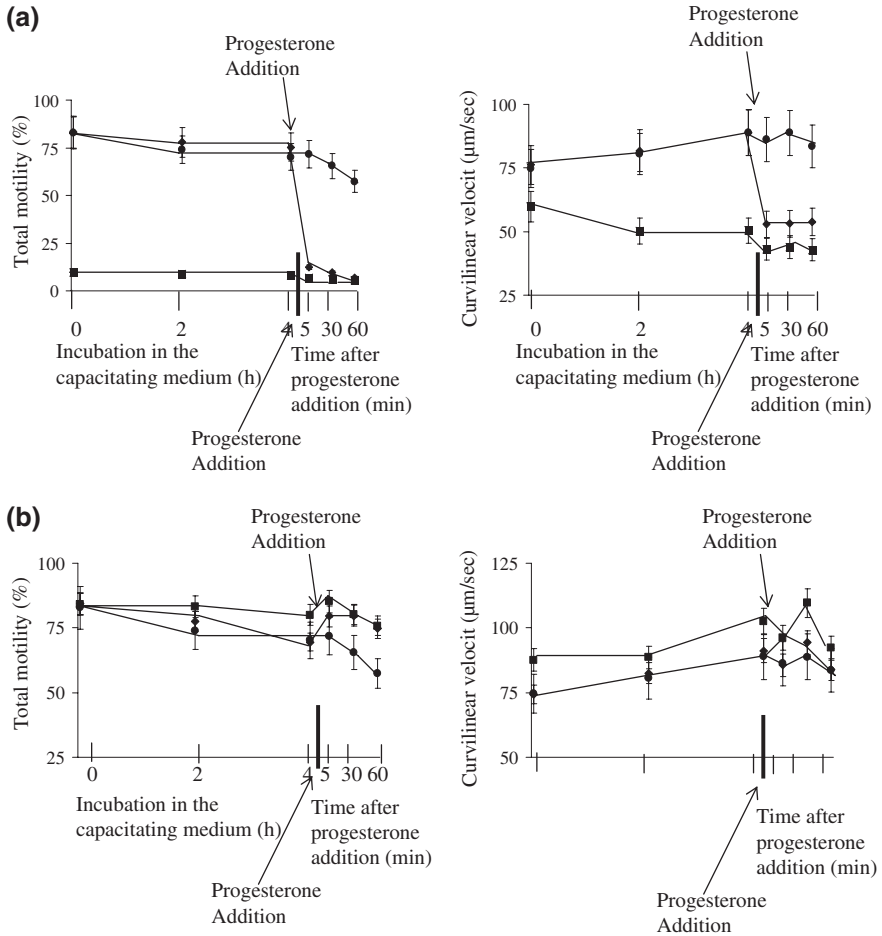


**Fig. 2.2** Ultrastructural image of boar sperm mitochondria. The low development of inner cristae is noticeable (*asterisks*). *BM* inner mitochondrial membrane. *P* cell membrane. *A* axoneme. *FD* dense fibres. *GP* peripheral granules. From Bonet et al. (2000)



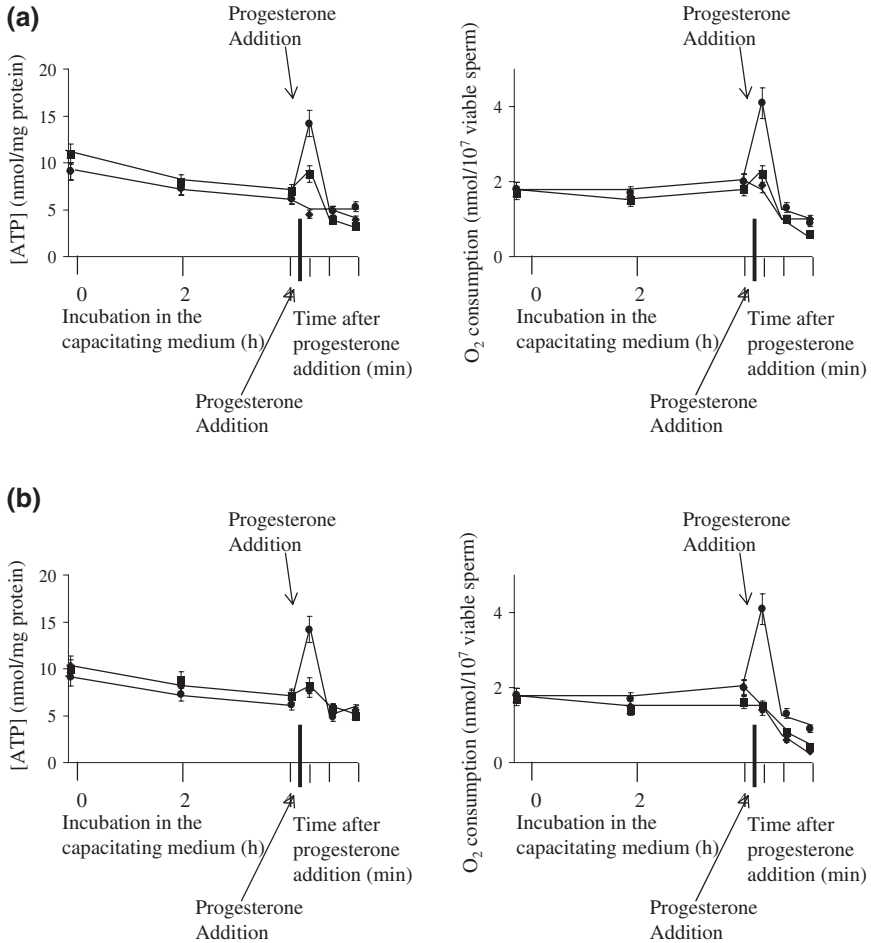
the low efficiency of boar sperm mitochondria as an energy synthesizer. However, this does not preclude that mitochondria-originated energy would not be important for boar sperm function. Thus, the achievement of a feasible, progesterone-induced “in vitro” acrosome reaction is concomitant with a sudden and intense peak of the  $O_2$  consumption rate and also of intracellular ATP levels (Ramíó-Lluch et al. 2011). This peak is not present in conditions in which progesterone-induced acrosome reaction is prevented (unpublished data from our laboratory), indicating thus a close relationship between mitochondria-generated energy and the achievement of the acrosome reaction, despite the low energy efficiency of these organelles.

However, the fact that mitochondrial respiration seems to be important only in specific moments of the boar sperm’s lifespan does not necessarily indicate that boar sperm mitochondria are only important in this aspect. In fact, mitochondria have many more roles than mere energy-producing factories. It is well known that mitochondria play a key role in eukaryotic cells in the control of other highly important aspects of eukaryotic cell function, such as the modulation of apoptosis and the control of calcium metabolism. Thus, it is likely that the most important functions of boar sperm mitochondria would be linked to the control of other cellular functional aspects rather than to energy management. In this regard, unpublished results from our laboratory strongly indicate that the incubation of boar sperm in a capacitation medium in the presence of oligomycin A, a specific inhibitor of the electronic chain-to-chemiosmosis step (Chappell and Greville 1961), immobilizes boar sperm and prevents them from achieving “in vitro” capacitation, without modifying either the rhythm of  $O_2$  production or the intracellular ATP



**Fig. 2.3** Percentages of total motility and curvilinear velocity values of boar sperm subjected to “in vitro” capacitation and subsequent, progesterone-induced “in vitro” acrosome reaction in a capacitation medium with or without the presence of either oligomycin A or Ca<sup>2+</sup> and EGTA. (a) spermatozoa incubated in a medium with or without oligomycin A. (b) spermatozoa incubated in a standard capacitation medium or a medium without Ca<sup>2+</sup> and with 2 mM EGTA added. ● sperm incubated in a standard capacitation medium. ■ sperm incubated in a medium with oligomycin A or in a medium without Ca<sup>2+</sup> and with 2 mM EGTA added. ◆ sperm incubated in a standard medium for 4 h and afterwards simultaneously with progesterone and either oligomycin A or 2 mM EGTA added. Results are mean ± S.E.M. for 7 different experiments. Unpublished results

levels (Figs. 2.3 and 2.4 and data not shown). On the contrary, the incubation of boar sperm in a capacitation medium without calcium induces an increase in the velocity parameters of these cells that was complementary to the already observed bicarbonate-induced, protein kinase A-modulated motility activation (Aparicio



**Fig. 2.4** Rates of O<sub>2</sub> production and intracellular ATP levels of boar sperm subjected to “in vitro” capacitation and subsequent, progesterone-induced “in vitro” acrosome reaction in a capacitation medium with or without the presence of either oligomycin A or Ca<sup>2+</sup> and EGTA. (a) spermatozoon incubated in a medium with or without oligomycin A. (b) spermatozoa incubated in a standard capacitation medium or a medium without Ca<sup>2+</sup> and with 2 mM EGTA added. ● sperm incubated in a standard capacitation medium. ■ sperm incubated in a medium with oligomycin A or in a medium without Ca<sup>2+</sup> and with 2 mM EGTA added. ◆ sperm incubated in a standard medium for 4 h and afterwards simultaneously with progesterone and either oligomycin A or 2 mM EGTA added. Results are mean ± S.E.M. for 7 different experiments. Unpublished results

et al. 2007; Harayama and Nakamura 2008; Kaneto et al. 2008). Notwithstanding, the lack of extracellular calcium prevents the achievement of capacitation (Fig. 2.3 and data not shown). This effect linked to the lack of extracellular calcium, however, is carried out without changes in either the rate of O<sub>2</sub> production or the

intracellular ATP levels (Fig. 2.4 and data not shown). Thus, these results clearly indicate that mitochondria play an important regulatory role in the control of functional aspects such as motility patterns and the achievement of “in vitro” capacitation by ways that are not directly linked to energy production, but to the control of intracellular reductive potential and intracellular calcium storage. This opens a new perspective in the way in which investigators should approach the understanding of the role played by mitochondria in the control of sperm function. However, a great deal of more work is needed in order to achieve a thorough insight into this complex phenomenon.

## 2.4 Sperm Subpopulations: Do they have a Definite Biological Role?

In a seminal article published in 1996 within the framework of the Third International Conference on Boar Semen Preservation, Holt described the presence of a specific subpopulation structure in boar ejaculates when sperm motility of these ejaculates was analyzed by using a computerized, automatized system (CASA system; see Holt 1996). In the same article, Holt described how these subpopulations would change according to the presence of several stimuli like bicarbonate in the dilution medium, thus suggesting a functional role for this subpopulation structure in boar ejaculate (Holt 1996). Since then, an increasingly large number of papers have described the presence of a similar subpopulation structure, not only in boar (Holt 1996; Abaigar et al. 1999), but also in other mammalian species, such as gazelle (Abaigar et al. 1999), horse (Quintero-Moreno et al. 2003), donkey (Miró et al. 2005), dog (Dorado et al. 2011), rabbit (Quintero-Moreno et al. 2007), deer (Martinez-Pastor et al. 2005), bovine (Muiño et al. 2008), and ovine (Rodríguez-Gil et al. 2007). This wide spectrum seems to indicate that, as a common characteristic, mammalian ejaculates have a subpopulation structure of their sperm. Moreover, these subpopulations have been observed not only when analyzing motility, but also after the observation of other ejaculate characteristics like sperm morphology (Rubio-Guillén et al. 2007), and even midpiece mitochondria activity through JC-1 stain (Ramíó-Lluch et al. 2011). These findings further reinforce the existence of a subpopulation structure inside mammalian ejaculates.

Taking into account the existence of sperm subpopulations, a highly important question is raised regarding this structure; what is the real, biological role of a subpopulation structure in mammalian ejaculates? This is currently an open question, and we can only speculate on this point. Notwithstanding, and centering on boar spermatozoa, we have enough information to establish some basic, preparative principles. In this regard, no definitive relationship has been established between subpopulations and “in vivo” fertility in a pig farm entourage (Quintero-Moreno et al. 2004). On the contrary, a subtle although significant relationship has been established between a specific subpopulation structure and the ability of boar spermatozoa to resist cryodamage after standard freezing-thawing procedure (Thurston

et al. 2001; Flores et al. 2009). This indicates that the capacity for resistance of boar ejaculates to freezing-thawing is related in some way with the specific subpopulation structure that this ejaculates presents before freezing. This feature could be related to specific functional aspects of boar sperm biology. In this respect, Satake et al. (2006) have observed that the interaction of boar sperm with oviductal proteins is related to the sensitivity of each spermatozoon to respond to bicarbonate stimulation. This result links the specific sensitivity of each boar sperm when undergoing capacitation to its ability to reach oocytes, thus opening the door to the presence of separate sperm subpopulations with different abilities to reach capacitation and subsequent sperm-oocyte interaction. These differences in sensitivity have also been observed by our laboratory after conducting “in vitro” capacitation and subsequent, progesterone-induced “in vitro” acrosome reaction experiments. In these experiments, the achievement of feasible “in vitro” capacitation and subsequent “in vitro” acrosome reaction has been linked to specific changes in the boar sperm subpopulation structure, especially affecting those sperm that present higher velocity characteristics at the start of incubation in the capacitation medium (Ramió et al. 2008). This seems to indicate that those sperm that show higher velocity characteristics in an ejaculate are prone to achieve feasible “in vitro” capacitation and further acrosome reaction. Interestingly, analysis of mitochondrial activity through JC-1 stain has shown not only the presence of a subpopulation structure based on boar sperm mitochondria activity, but also specific changes in this subpopulation structure after “in vitro” capacitation and acrosome reaction, with higher changes in those sperm that show the lowest mitochondrial activity in freshly obtained ejaculates (Ramió-Lluch et al. 2011). These results indicate the existence of a close relationship between the specific motility characteristics of specific boar sperm and their mitochondrial activity. This close relationship between boar sperm motility and their mitochondrial activity status would then suggest a biological role in the subpopulation structure. This role would be related to the separate ability of sperm from each subpopulation to present a specific functional activity, resulting in separate mitochondria activity profiles. In turn, these specific functional changes would be related to the ability of each sperm to yield changes such as those related to the achievement of capacitation, thus linking the subpopulation structure with the fertilizing ability of an ejaculate. However, a great deal of work is necessary in order to elucidate this important question. In any case, it is obvious that boar sperm quality analysis would have to be modified in order to introduce the subpopulation concept if practitioners sought to obtain optimal information regarding the specific quality of the analyzed ejaculate.

## 2.5 Conclusion

In the past few years, an increasing amount of information has been gathered to alter our overall vision of boar sperm biology. Therefore, the old image of a simple, straightforward cell must be abandoned to make way for a concept involving a highly complex cell, which is able to adapt its resources to the changes in the

environment through a myriad of complex mechanisms regulated with very fine and sensitive systems. Moreover, the concept of a complex, subpopulation structure of boar ejaculates must be considered as an important characteristic involved in the fertilizing ability of boar sperm, and semen quality analysis should be designed in order to incorporate this biological characteristic.

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