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Review Article

Bovine Sperm Capacitation: Physiological Changes and Evaluations

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Abstract

Sperm capacitation is defined by biochemical modifications that spermatozoon must pass to become capable to allow them to acrosome react upon exposure to the zona pellucida, cumulus cells, to fertilize an oocyte, therefore an extremely important event into the reproduction. It is known that the capacitation is a process that involves a series of molecular factors, which reflect not only in biochemical modifications such as biophysical characteristics, such as hyperactivation of motility. However, some important characteristics such as how to evaluate this physiological state of the cell remain controversial. In general, several laboratory assays have been developed in order to obtain reliable results in sperm evaluations, increasing the quality and reliability of the semen used in assisted reproduction techniques. These evaluations have allowed us to identified physiological characteristics such as sperm capacitation, as well as damages in specific compartments and organelles, which are not detected in the routine analyzes. Among them, the use of fluorescent probes and their detection in epifluorescence microscopy or flow cytometry have become an important tool when an accurate evaluation is necessary. In the case of evaluations that predict the status of sperm capacitation, these are more complex than other evaluations, because they are related to changes in the intracellular metabolism of spermatozoa. The fluorescence technique based on chlortetracycline (CTC) or Merocyanine 540 is used to estimate whether sperm passes through the capacitation process. This review focuses on the physiological changes process that occurs during capacitation process and how it is evaluated this cell physiological status.

INTRODUCTION

After ejaculation, the spermatozoa are not capable to fertilize an oocyte. Sperm capacitation is the set of events that allows the sperm to fertilize an oocyte [1,2].

The fertilization ability of sperm require through two phases of sperm maturation. The first occurs in the epididymis, where physiological changes occur in the lipid and protein content of spermatozoa [3,4]. Among the main constituents that make up the epididymal environment are several proteins, such as osteopontin, mannosidase, galactosidase, lactoferrin and clusterin [5]. The sperm motility is a characteristic that the cell acquires during maturation, at the epididymis transit. The cells begin some vibratory movements at the body of epididymis, and at the tail it's possible found sperm with motility [1,5,6]. These factors associated with a differentiated environment of the epididymis ensure the sperm maturation, maintenance of sperm viability, and preservation of fertilization capacity for several weeks [6].

After epididymal maturation, sperm cell requires others surface modifications that occurs inside female genital tract, specifically in the oviduct. In contact with oviduct, some molecules how glycosaminoglycans (GAGs) such as heparin for bovine, sodium bicarbonate ($NaHCO_3$), calcium (Ca^{2+}) and bovine serum albumin (BSA), interact with the spermatozoa, modifying their membrane and triggering the process of capacitation [1,7,8]. Currently the most widely used probes to evaluate cells in the capacitation stage are Merocyanine 540 (M540) and Chlortetracycline Hydrochloride (CTC), which may or may not be associated with other evaluations such as acrosomal integrity. Hyperactivity associated with other factors may also be indicative of sperm capacitation [9].

The purpose of this short review is to present how capacitation occurs and discussed about the two most used evaluations methods.

Capacitation

The process of sperm capacitation is defined by a series of

physiological changes, involving the removal and alterations of substances that stabilize sperm membrane. These physiological changes occur when spermatozoa comes in contact with the seminal plasma, in the reproductive tract of the female, specifically in the oviduct, or in vitro, when incubated in specific medium [1,7,10-12].

Among the components related to the capacitation process are BSA, $NaHCO_3$, Ca^{2+} and heparin [3,4,10,13]. Follicular fluid and/or oviduct secretions play a role in capacitating or inducing the acrosome reaction in sperm. Follicular fluid and oviduct secretions are rich in GAGs [15-17]. Some of these molecules are present in the seminal plasma and/or oviduct fluid, such as BSA, and may act as cholesterol acceptor from the sperm plasma membrane, promoting capacitation [2,3,8,18].

Initially, it occurs changes mediated by the action of bicarbonate, which alter the fluidity of the membrane, inducing asymmetry of the phospholipids. Albumin-mediated plasma membrane cholesterol efflux is a later event in sperm capacitation, resulting in plasma membrane scrambling and Ca^{2+} influx [8,14]. Due to the increased levels of cAMP, occurs activate of protein Kinase A (PKAs), cAMP-dependent. Therefore, indirectly induce protein tyrosine phosphorylation. Bicarbonate also induces PKA-dependent. Thus, changes in the lipid architecture of the sperm plasma membrane, thereby the scrambling of the plasma membrane occur [3,19,20].

Acrosome Reaction

The final of the capacitation modifications occurs by the acrosomal reaction. It is the moment of fusion between the external membrane and the plasma membrane, in the inner portion of the spermatozoa head, allowing the release of hydrolytic enzymes such as hyaluronidase and acrosin that will digest the zona pellucida. The communication of sperm proteins and oocyte glycoproteins, at the presence of follicular fluid and secretions present in oviduct, rich in GAGs, signaling for calcium uptake and induction of the reaction [21-24].

For this, it is important an intact acrosome, so that penetration of the spermatozoon occurs through the zona pellucida of the oocyte [25]. In addition, there is a need for reorganization of membrane proteins and lipids, changes in displacement characteristics and changes in the metabolic activity of the spermatozoa [11,22-26].

Capacitation evaluations

For capacitation status for assessments, two probes can be used: the lipophilic probe M540, and the calcium hydrochloride of chlortetracycline, popularly known as CTC.

Due to the physiological complexity of the molecules that trigger the capacitation events, changes in the intracellular metabolism of spermatozoa are more difficult to evaluate [27,28], than others parameters. The use of CTC has been proposed as a rapid method for evaluating sperm capacitation. During capacitation, changes in CTC fluorescence distribution are observed in response to fluid changes in sperm plasma membrane and Ca^{2+} influx (final stage of capacitation) [29], as a Ca^{2+} chelator, CTC indicates the location of Ca^{2+} [30]. Three fluorescence patterns were defined: bright fluorescence over the whole head (uncapacitated cells); fluorescence-free band in the post acrosomal region (capacitated cells); and full fluorescence over the whole head except for a thin, bright band of fluorescence along the equatorial region (acrosome-reacted cells) [30,31].

M540 is a lipophilic molecule composed of negative charge due to its sulfonamide group. It is therefore impermeable to intact cells, can be bound only to the external leaflet of their plasma membranes, with an affinity that appears to reflect degree of lipid disorder. Therefore, lipophilic this molecule acts as a marker of the phospholipid disorder level of the plasma membrane lipid bilayer. The presence of a fluorescence intensity is a cell with phospholipid scrambling [18,32].

Although it has been used in the last two decades by several authors and species such as stallion [14], boar [3,18] and bovine [33,34] in studies that associate or not with other assessments of capacitation status, there are still some doubts regarding the accuracy and efficacy of the technique. Because some studies hypothesis that lipid disorder of the plasma membrane as a reversible event that may occur due to capacity process or due to cell death [18].

In our opinion, theses mistakes about scrambling caused by beginning capacitation or cell death is still a bit confusing. Since all cells that undergo capacitation and not bound at zona pellucida will die. The pathways that spermatozoa die by capacitation process appear be different that others causes of death, for example necrosis or apoptosis. Maybe, this problem can be diminished using associations with other probes that stain death cells, such as YoPro1. Thus, is possible the separation of the groups of dead cells and dead cells with scrambling.

FINAL CONSIDERATIONS

Due the complexity of a series of events, that involves capacitation process, assays are not commonly and many doubts about evaluations are constant. Maybe, the junction of factors and evaluations, such as capacitation, acrosome reaction and hyperactivation can be result in researches more reliable.

Although CTC is a pioneering and reliable technique, that can show differentiation between capacitated or uncapacitated cells, as also cells with intact and reacted acrosome, unfortunately the technique has the disadvantage that it cannot be used for evaluations in the flow cytometry. Consequently reaching subjective data, when compared to flow cytometry. Moreover, the fixation that the protocol require can cause undesirable features such as artifacts, cell and acrosomal degeneration.

In addition, the influence of Ca^{2+} influx is not an early event in the capacitation process, whereas the destabilization of plasma membrane is the first characteristic of capacitation [35,13,14]. *In vivo*, Ca^{2+} is physiologically present in the female reproductive tract. However, many *in vitro* assays do not have calcium in their medium, making CTC unfeasible to assess training in these cases. The M540 seems to have the advantage of detecting fluidity

changes, being much more precise and safe when compared to CTC.

The physiological characteristics that differentiate the two evaluations were demonstrated in a study by Rathi et al. [14], where it was possible to observe that M540 detects spermatozoa in the initial stage of the capacitation, approximately 30 minutes, while the CTC is able to identify changes just after incubation in 5 min with HCO_3, in Ca^{2+} supplemented medium. It was possible to observe changes both in M540 and in CTC at 30 minutes of incubation, which can be justified because the CTC technique is calcium dependent.

Using a medium that detects a highly specific mineral such as calcium is not always advantageous. Since not only calcium is involved in the training process with also cholesterol, sodium bicarbonate, ands GAG's.

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