Chapter 8

In Vitro Embryo Production in the Pig

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Abstract

Pigs show economic importance and physiological similarities to humans. For this reason, the *in vitro* production (IVP) of swine embryos is an important biotechnological tool. However, despite the advances in IVP of mice and cattle, the progress in this specie is relatively slow. *In vitro* pig embryos are obtained by a series of integrated and efficient manipulations of follicular oocytes. The IVP process includes four technical steps: (1) Oocyte collection (ovaries from slaughterhouse), (2) *In vitro* maturation (IVM) of immature oocytes, (3) *In vitro* fertilization (IVF) of matured oocytes and (4) *In vitro* culture (IVC) of zygotes. In this chapter we have discussed and provided different steps of IVP and general notions of the physiological processes, as well as recent advances and obstacles of IVP of embryos in pigs.

Introduction

Swine breeding is an important economic activity in the world. Data shows that, in European Union (EU), swine meat represents 9.0 % of the total agricultural output and, from 2009 to 2014, it has increased by 3.6 %, while bovine meat output has decreased by 5.7 %. The production in the European Union was driven by the export surge of swine meat to China, low feed prices and a higher number of breeding cows, reaching 22.9 million tons [1].

Furthermore, pigs show physiological similarities to humans, that has ended up rising the economic interest in swine biotechnologies by both biomedical and swine industries. The interest has created an increased desire for new technologies as well as an urge for implementation of the existing ones [2]. At this point, the *in vitro* production (IVP) of swine embryos is interesting to researchers. These animals can be used as human biomodels and for creating genetically modified animals as potential donors of tissues and organs for xenotransplantation [3].
Reproduction Biotechnology in Farm Animals

The first approach using IVP technology in pigs was established in 1986 [4] from in vitro fertilization (IVF) of in vivo matured oocytes. After that, in 1989, the first production of piglets by IVP was reported [5]. In that study, in vitro matured and in vitro fertilized oocytes produced 2-cell to 4-cell embryos after 44 h post-insemination (post insemination - pi). Since then, studies have shown that successful large-scale IVP of porcine embryos can provide viable embryos more efficiently, with less cost and in less time, when compared to the surgical collection of in vivo derived embryos from sows. For in vitro culture (IVC), procedures have improved, however, for in vitro maturation (IVM) and IVF systems still have several unsolved problems, including imbalance of nuclear and cytoplasmic maturation and polyspermy [6].

Porcine in vitro embryos are obtained from a series of integrated and efficient techniques by manipulation of follicular oocytes in vitro. The process includes four technical steps: (1) oocyte collection (ovaries from slaughterhouse), (2) IVM of immature oocytes, (3) IVF of matured oocytes and (4) IVC of zygotes. According to the currently applied protocols in immature porcine oocytes, there is a lot of variation in the results, but around 20–30% develop to blastocyst stage [7,8]. However, a substantial proportion of embryo polyploidy is a problem observed in porcine IVP [6].

In this chapter we will discuss and provide different steps of IVP and general notions of the physiological processes, as well as recent advances and obstacles observed during in vitro maturation, fertilization and culture of pig embryos.

Oocyte Collection and In Vitro Maturation

Collection of good-quality oocytes is the first step for in vitro embryo production. There are two sources to collect oocytes in pigs: surgical procedures or, more commonly, ovaries from slaughterhouses.
The ovaries, obtained from abattoir, should be transported to the laboratory within 2h, immersed in 0.9% NaCl or phosphate-buffered saline (PBS) solution, supplemented with 75 µg/mL potassium penicillin G and 50 mg/mL streptomycin sulfate, at 30-35°C. Only follicles between 3–6 mm should be aspirated by an 18-gauge needle, attached to a 10-mL disposable syringe, or connected to Falcon tube, under controlled vacuum (30 mm Hg). Then, cumulus oocyte complexes (COCs) with a uniform ooplasm and compact cumulus cell mass should be selected under a stereomicroscope for IVM [9,10].

Before moving to the next step of IVP, attention must be paid to the quality of culture media used. These solutions are of critical importance and will reflect on subsequent embryo development. In that perspective, the improvement of media used for IVM, IVF and IVC brings reliability and reproducibility of results among different laboratories [11]. Another key factor that can affect the success of culture media is the osmolality [12]. It was proposed that the osmolality of IVM, IVF and IVC media should remain stable during incubation [13]. The osmotic stresses can damage DNA and affect DNA replication, DNA transcription and mRNA translation, leading to cellular disturbance [14]. Porcine oocytes cultured with hyperosmotic solutions (>300 mOsm/kg) lose the ability to develop into blastocyst in one-half of oocytes evaluated, showing the importance of osmolality control [15].

Low temperature is another variable that can be harmful to oocytes. Temperature reduction is associated to ruptures of the cytoskeleton as it also decreases cellular metabolism [16,17]. The ideal temperature for culture in pigs is around 38.5°C [18].

After oocyte collection, the next crucial step is IVM. The oocyte maturation involves cell changes that transform an oocyte unable to progress, to a one able to support the events of fertilization and embryo development. During the process, immature COCs undergoes meiosis, reducing their chromosomes quantity to half, extrusion of the first polar body, reorganization of all organelles, to finally receive the spermatozoa [19].
For IVM of pig oocytes, several basic culture medium types have been used, including North Carolina State University (NCSU) medium [20], modified tissue culture medium (TCM 199) and modified Tyrode’s medium, containing lactate and pyruvate (mTLP) [21]. These media are generally supplemented with porcine follicular fluid (pFF), or serum. However, pFF is not available commercially and it shows variability in its properties, depending on how it’s prepared. In contrast, fetal bovine serum (FBS) is commercially available, with quality assured by the supplier, showing several advantageous components, such as low levels of antibodies and numerous growth factors [22,23]. The oocytes matured in NCSU-37 medium, supplemented with FBS, supported blastocysts to develop into piglets after IVF and IVC [24].

Supplementation of different hormones, such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), pregnant mare serum gonadotrophin (PMSG), human chorionic gonadotropin (hCG), estradiol-17β, leptin and relaxin, in IVM medium, has shown beneficial effects on oocyte maturation. Moreover, growth factors and other substances, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), growth differentiation factor 9 (GDF-9), Transforming growth factor (TGF)-α, epidermal growth factor (EGF), EGF-like peptides and L-carnitine, also show improvement of oocyte maturation [22].

A problem usually observed in porcine IVM oocytes is the reduced developmental ability as compared to their in vivo matured counterparts, suggesting cytoplasmic insufficiency (Nagai et al., 2006). However, the cytoplasmic maturation of IVM oocytes could be improved by protecting oocytes from oxidative stress, caused by reactive oxygen species (ROS) [25].

Glutathione (GSH) is known to be an important intracellular factor that can control cellular levels of ROS and protect the oocytes against the damaging effects of oxidative stress [26]. Therefore, the ideal IVM medium should be capable to enhance the cytoplasmic GSH contents of matured oocytes. Stimulating synthesis of intracel-
lular GSH is the main beneficial effect of IVM medium supplementa-
tion with cysteine and EGF [27,28].

Zinc is an essential trace mineral required for normal fetal
growth and development. And also, zinc is known to influence preg-
nancy, embryonic development, and fetal survival in mammals [29].
The zinc supplementation in IVM medium was recently assessment
[30]. The addition of 0.8 µg/mL zinc increased the intracellular GSH
concentration, reducing the ROS level, during porcine IVM, with
beneficial effect on blastocyst formation.

To achieve an ideal IVM, immature COCs should be incubated
in maturation medium, chosen according to protocols, and cultured
for around 44 hours, at 38.8 °C, under an atmosphere air of 5% CO2,
with maximum humidity [9].

**In Vitro Fertilization**

*In vitro* fertilization is a crucial step in the embryo IVP pro-
cedure. However, several variables are involved in this process. For
proper IVF, fresh or cryopreserved semen must be prepared before
incubation with oocytes. Seminal plasma and/or extender contain
components that function as decapacitation factors and must be re-
moved before co-incubation with oocytes [31]. Thus, before sperm
capacitation for IVF, boar spermatozoa have conventionally been
washed to separate them from seminal plasma and extender by simple
centrifugation [32,33]. It appears to resist a high g-force (2400 x g), for
a relatively short centrifugation time (3 minutes) [34].

Percoll gradient centrifugation has been used and showed higher
in vitro penetration rates and increased cleavage and blastocyst for-
mation rates after IVF [35,36,37]. Another method of sperm separa-
tion is swim-up. The procedure has been successfully used to isolate
a highly motile sperm population [38]. Moreover, beyond separating
good sperm for IVF, it has been reported that the modified swim-up
method, based on general sperm swim-up technique, could reduce
the occurrence of polyspermy in pig oocytes, during IVF. It increases
the outcome of normal karyotypes, leading to an improved development potential in porcine embryos [39].

At the end of pig oocyte maturation, cumulus oocyte-complexes (COCs) should be separated from cumulus cells, in a process called oocyte denudation. The COCs can be denuded by pipetting or vortexing and, regardless the methodology chosen, both procedures result in similar final rates of embryo development. However, researchers found that the denuding procedure should be used with care, concerning: induction of oocyte damage, changes in the position of the first polar body regarding the metaphase spindle, the spindle pattern, and cytoplasmic maturation [10].

For IVF procedure, the oocytes should be incubated with a sperm concentration defined by IVF protocol. Studies using 1 to 4.5 x 10^5 cells/mL showed good results [9,10]. It is important to notice that cell culture incubator at 38.8 °C and a humidified atmosphere of 5% CO2, for a period of 18-24 hours, is the ideal environment to perform IVF.

It is not easy to choose one among all the culture media that have already been tested for IVF in pigs. However, once selected, the basal fertilization medium is always supplemented with different additives to improve the results. It is the selection of these molecules that will designate the nature of the medium: chemically defined or undefined. Thus, there are some options of different media that can be used. Table 1, below, describes the main composition of basal IVF media used for swine culture [revised by 31].
**Table 1:** Composition of basal IVF media used in the pig.

<table>
<thead>
<tr>
<th>Component (mM)</th>
<th>mTBM</th>
<th>mTALP</th>
<th>mTCM-199</th>
<th>PGM</th>
<th>PGMtac4</th>
</tr>
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<tbody>
<tr>
<td>NaCl</td>
<td>113.10</td>
<td>114.06</td>
<td>116.35</td>
<td>108.00</td>
<td>108.00</td>
</tr>
<tr>
<td>KCl</td>
<td>3.00</td>
<td>3.20</td>
<td>5.36</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>MgCl$_2$●6H$_2$O</td>
<td>-</td>
<td>0.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MgSO$_4$</td>
<td>-</td>
<td>-</td>
<td>0.81</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MgSO$_4$●7H$_2$O</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Na-lactate 60% syrup. (mL/L)</td>
<td>-</td>
<td>1.85</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NaH$_2$PO$_4$</td>
<td>-</td>
<td>0.35</td>
<td>1.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>11.00</td>
<td>5.00</td>
<td>3.05</td>
<td>1.00</td>
<td>-</td>
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<td>NaHCO$_3$</td>
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<td>25.07</td>
<td>26.19</td>
<td>25.07</td>
<td>25.00</td>
</tr>
<tr>
<td>Caffeine</td>
<td>1.00</td>
<td>2.00</td>
<td>5.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ca-(lactate)$_2$</td>
<td>-</td>
<td>-</td>
<td>2.92</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ca-(lactate)$_2$●5H$_2$O</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.50</td>
<td>4.00</td>
</tr>
<tr>
<td>Tris</td>
<td>20.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Na-pyruvate</td>
<td>5.00</td>
<td>0.11</td>
<td>0.91</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>CaCl$_2$●2H$_2$O</td>
<td>7.50</td>
<td>4.70</td>
<td>1.80</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>-</td>
<td>-</td>
<td>12.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Polyvinyl-Alcohol (mg/mL)</td>
<td>-</td>
<td>1.00</td>
<td>-</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Theophylline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.50</td>
</tr>
<tr>
<td>Adenosine (µM)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
</tr>
<tr>
<td>Cysteine (µM)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.25</td>
</tr>
<tr>
<td>Gentamicin (mg/mL)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Penicillin G/ streptomycin</td>
<td>-</td>
<td>-</td>
<td>0.17/0.07</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amikacin sulfate (mg/mL)</td>
<td>-</td>
<td>0.10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BSA (mg/ml)</td>
<td>1.00</td>
<td>3.00</td>
<td>4.00</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*References: [32] [40] [41] [42] [42]*

*mTALP: modified Tyrode’s albumin lactate pyruvate; mTBM, modified Tris-buffered medium;*
mTCM-199, modified tissue culture medium-199; PGM, porcine gamete medium; PGMtac, porcine gamete medium theophylline–adenosine–cysteine; mTCM-199: partial listing of components of TCM-199 with Earle’s salts and L-glutamine (cat. no. M-5017; Sigma). There are different supplementations of TCM.

The success of embryo development to the blastocyst stage during IVP relies on oocyte cytoplasm to facilitate normal fertilization and subsequent embryo development [43]. The process by which immature oocytes acquire that ability, during IVM, is frequently called cytoplasmic maturation. High polyspermy rates and low blastocyst developmental rates are considered to be the two major obstacles in porcine IVP [6]. Those obstacles may be related to insufficient cytoplasmic maturation during IVM.

During IVF process, penetration of the oocyte cytoplasm by more than one spermatozoa may occur and is considered a pathologic condition, observed in placental mammals, called polyspermy. Usually, it causes early death of the embryo. Particularly in pigs, this defect affects approximately 50% of in vitro fertilized oocytes [44]. Since the release of the first studies about IVM-IVF in pigs, problems of polyspermy and male pronucleus formation were often observed. Researchers thought that polyspermy could be simply resolved by reducing the numbers of sperm available to the mature oocyte, during IVF. However, that approach has been proved wrong and it does not provide a solution [45].

Physiologically, the oocyte fertilization in mammals induces cortical granules (CG) exocytosis, which is initiated by calcium oscillations during sperm penetration [46]. Moreover, it induces the fusion of the CG to the ooplasm and the exocytosis of their content into the perivitelline space, which modifies the oocyte plasma membrane and the zona pellucida (ZP), turning the oocyte refractory to additional sperm binding and penetration [47]. Other mechanisms occurring inside the oviduct, reducing the mass arrival of spermatozoa in the
vicinity of the oocyte, also have been proposed as contributing to the blockage of polyspermy [48].

The final maturation of the oocyte in the oviductal environment is suggested as a necessary step for successful fertilization and embryo development [49,50]. Indeed, many existing studies support a functional role of the oviduct and its secretions (which are rich in estrus-associated glycoproteins) during fertilization. They regulate processes such as sperm–ZP binding, establishment of species-specific ZP barriers and early embryonic development [51,52].

In that perspective, the efficiency of the incorporation of oviduct fluid (OF) in IVF medium have been tested. Recent studies show that the incubation of matured oocytes with pure OF for 30 minutes, before IVF, or 10% OF, during IVF, decreases the number of ZP sperm-bound and the incidence of polyspermy, regardless of sperm concentration [53].

Those findings can be explained by the presence of important contents in OF that contribute to the regulation of fertilization. Several proteins in the OF can bind to the ZP, modifying both its protein and carbohydrate composition [53]. Moreover, hypothesis have been raised stating that the sperm-recognizing labels coming from OF can be incorporated in the ZP, helping the selection of specific subpopulations of capacitated spermatozoa, even though, the final number of spermatozoa around the oocyte, at the fertilization time, is reduced in vivo [44].

The presence of OF, before or during IVF, allows to increase the sperm concentration, without affecting the monospermy rate [44]. The exposure of oocytes to pure OF, before IVF, or diluted OF (10%), during IVF, improved the efficiency of production of monospermic zygotes. The most effective combination for achieving a higher monospermic zygote production in IVF is: sperm concentration of $4.5 \times 10^5$ cells/mL and oocyte exposure to OF, either before or during IVF [9].
The OF can be obtained by selecting and treating collected genital tracts from slaughterhouses. Briefly, based on ovarian morphology of both ovaries, the oviducts at late follicular phase should be selected by established criteria [54]. Thereafter, these oviducts should be quickly washed, once, with 70% ethanol solution and, twice, with Dulbecco’s PBS, and then, transferred to cold Petri dishes (on ice), to be dissected free of surrounding tissues. The oviducts, flushed with 500 mL of PBS, should be inserted into the ampulla tip for washing the lumen. That procedure should be repeated with 10 oviducts. The same fluid recovered in the first oviduct should be reused to wash the next one. Finally, the oviductal flush should be centrifuged at 7000 x g, for 10 minutes, at 4 °C, to remove cellular debris. Then, the supernatant must be immediately stored at -20 °C, until its use [9].

Another alternative method to obtain less polyspermic fertilization and to achieve higher developmental competence of in vitro-matured oocytes is the use of modulators of cAMP during pig IVM. One of these modulators is dibutyryl cAMP sodium salt (dbcAMP), which has been used during IVM to increase cAMP levels, postpone meiosis, and consequently synchronizes nuclear and cytoplasmic maturation of the oocytes [55,56]. The results, when using that substance, show that the addition of dbcAMP in the medium, during the first 22 hours of IVM, induced a lower penetration rate followed by a lower polyspermy rate after IVF. Ultimately, it results in a higher percentage of fertilized zygotes in pigs [55].

**In Vitro Culture**

IVC is the last step of embryo IVP in pigs. Revising the entire process, immature COC are obtained from ovaries (1), then, *in vitro* matured (2) and, *in vitro* fertilized (3). The zygotes will undergo cleavage (4), will develop into morulae (5) and, finally, will develop into a blastocyst (6). Figure 1 shows the schematic morphologies of embryo IVP.
IVC conditions are the main variables that can affect quality and yield of blastocyst development [57]. For ideal embryo transfer, the use of high-quality blastocysts (with numerous nuclei and lower apoptotic index) increases the rates of pregnancy success [58]. Thus, the media composition is one critical element to achieve excellent quality blastocysts. Usually, the IVC medium contains defined levels of different energy sources: glucose, calcium, lactate and pyruvate, as well as supplement vitamins [57].

Early-stage embryos are the ideal ones for transfer, especially because it minimizes in vitro exposure. However, that would preclude the assessment of embryonic developmental capacity. It was observed that the ideal culture time for porcine embryos is 5 to 6 days. When those embryos are cultured for 7-8 days, they show a lower quality and a higher apoptotic frequency [58,59]. That phenomenon can be

**Figure 1:** Schematic representation of stages of embryo development in pigs. COC: cumulus-oocyte complexes.
explained by the increase of the ROS (also called free radicals). High levels of ROS cause oxidative stress, decreasing trophectoderm (TE) cells in IVP blastocysts, which results in delayed post-implantation development [60,61].

In that perspective, the use of strategies to avoid increase of ROS must be pursued. Thus, the IVC performed in low oxygen levels (5% of O2, 5% of CO2 and 90% of N2) [62,63,64] may reduce the ROS levels. However, some researchers control only CO2 levels, without controlling oxygen levels (using 5% of CO2 in atmosphere) [65,66].

Media used for IVC can be classified according to their composition and divided into three groups: (1) simple media, originally developed for mouse embryos (for example: Simple Optimized Medium - SOM, Chatoz Ziomec Bavister-CZB); (2) media derived from the liquid composition of the oviduct (for example: Synthetic Oviductal Fluid - SOF, Human Tubal Fluid - HTF); and finally, (3) medium designed to culture of somatic cells (for example: Tissue Culture Medium - TCM199) [67,68].

In swine, the most commonly media used are: simple medium NCSU-23 (North Carolina State University) and oviduct liquid composition medium PZM-3 (Porcine Zygote Medium). The use of PMZ-3 supplement by FBS reducing the ROS levels, which leads to improved blastocyst development and hatching rates [65]. Table 2 shows the composition of these media [reviewed by 63].
Table 2: Composition of IVC media used for pig.

<table>
<thead>
<tr>
<th>Component</th>
<th>PZM-3</th>
<th>NCSU-23</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (mM)</td>
<td>108.00</td>
<td>108.73</td>
</tr>
<tr>
<td>KCL (mM)</td>
<td>10.00</td>
<td>4.78</td>
</tr>
<tr>
<td>CaCl₂,2H₂O (mM)</td>
<td>−</td>
<td>1.70</td>
</tr>
<tr>
<td>KH₂PO₄ (mM)</td>
<td>0.35</td>
<td>1.19</td>
</tr>
<tr>
<td>MgSO₄·7H₂O (mM)</td>
<td>0.40</td>
<td>1.19</td>
</tr>
<tr>
<td>NaHCO₃ (mM)</td>
<td>25.07</td>
<td>25.07</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>−</td>
<td>5.55</td>
</tr>
<tr>
<td>Na-Pyruvate (mM)</td>
<td>0.20</td>
<td>−</td>
</tr>
<tr>
<td>Ca-(lactate), 5H₂O (mM)</td>
<td>2.00</td>
<td>−</td>
</tr>
<tr>
<td>L-Glutamine (mM)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Taurine (mM)</td>
<td>−</td>
<td>7.00</td>
</tr>
<tr>
<td>Hypotaurine (mM)</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Basal Medium Eagle amino acids</td>
<td>20.00</td>
<td>−</td>
</tr>
<tr>
<td>Minimum Essential Medium</td>
<td>10.00</td>
<td>−</td>
</tr>
<tr>
<td>nonessential amino acids</td>
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<td></td>
</tr>
<tr>
<td>Gentamicin (mg/ml)</td>
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<td>0.05</td>
</tr>
<tr>
<td>Fatty acid-free BSA (mg/ml)</td>
<td>3.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Osmolarity (mOsm)</td>
<td>288±2</td>
<td>291±2</td>
</tr>
<tr>
<td>Ph</td>
<td>7.3±0.02</td>
<td>7.3±0.02</td>
</tr>
</tbody>
</table>

*NCSU-23: North Carolina State University; PZM-3: Porcine Zygote Medium.

Protocols used in porcine IVC shows variation among laboratories. A big challenge to be outpaced is ensure the reproducibility of protocols, and establishment of blastocyst rates. Table 3 shows the rates of blastocysts obtained by comparing the IVC in different atmospheres and media.
Table 3: Blastocysts rates in different media and atmosphere.

<table>
<thead>
<tr>
<th>Atmosphere</th>
<th>IVC medium</th>
<th>Blastocysts rates (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% CO₂</td>
<td>PZM-3</td>
<td>48.9±4.1*</td>
<td>[65]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35.5±3.2**</td>
<td>[65]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.2±0.5</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td>NCSU-23</td>
<td>61.7</td>
<td>[63]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34.2 ± 10.7</td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>59.6</td>
<td>[63]</td>
</tr>
<tr>
<td>5% O₂, 5% CO₂ and 90% N₂</td>
<td>PZM-3</td>
<td>24.0</td>
<td>[62]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>65.9</td>
<td>[63]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28.2± 3.3</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td>NCSU-23</td>
<td>32.6</td>
<td>[63]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.6</td>
<td>[64]</td>
</tr>
</tbody>
</table>

Data are the mean±SEM; Blastocyst rates: number of blastocyst/total of oocytes cultivated.
NCSU-23: North Carolina State University; PZM-3: Porcine Zygote Medium.
*IVC medium supplemented with fetal bovine serum (FBS).
**IVC medium without FBS.

Conclusion

In the present chapter, main steps of embryo IVP in pig were presented to understand the recent advances and barriers. Despite the biotechnological improvements, a high proportion of embryo polyploidy and variations of embryo development among laboratories is observed. However, recent strategies presented, such as, supplementation of oviductal fluid, Zinc, modulators of cAMP and incubation in low oxygen levels can improve the embryo IVP in pigs. Presently, further studies should be implemented to increase the production of good quality embryos capable of developing to term.
References


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