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In vitro embryo production in goats: Slaughterhouse and laparoscopic ovum pick up–derived oocytes have different kinetics and requirements regarding maturation media

Joanna Maria G. Souza-Fabjan [a,b,*], Yann Locatelli [a,c], Nicolas Duffard [a,c], Emilie Corbin [a], Jean-Luc Touzé [a], Christine Perreau [a], Jean François Beckers [d], Vicente José F. Freitas [b], Pascal Mermillod [a]

[a] *INRA, UMR7247, Physiologie de la Reproduction et des Comportements, INRA, CNRS, Université de Tours, Haras Nationaux, Nouzilly, France*
[b] *Laboratory of Physiology and Control of Reproduction, Veterinary School, State University of Ceará, Fortaleza, Ceara, Brazil*
[c] *Museum National d'Histoire Naturelle, Réserve de la Haute Touche, Obterre, France*
[d] *Laboratory of Endocrinology, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium*

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ABSTRACT

A total of 3427 goat oocytes were used in this study to identify possible differences during *in vitro* embryo production from slaughterhouse or laparoscopic ovum pick up (LOPU) oocytes. In experiment 1, one complex, one semi-defined, and one simplified IVM media were compared using slaughterhouse oocytes. In experiment 2, we checked the effect of oocyte origin (slaughterhouse or LOPU) on the kinetics of maturation (18 vs. 22 vs. 26 hours) when submitted to semi-defined or simplified media. In experiment 3, we determined the differences in embryo development between slaughterhouse and LOPU oocytes when submitted to both media and then to IVF or parthenogenetic activation (PA). Embryos from all groups were vitrified, and their viability evaluated *in vitro* after thawing. In experiment 1, no difference ($P > 0.05$) was detected among treatments for maturation rate (metaphase II [MII]; 88% on average), cleavage (72%), blastocyst from the initial number of cumulus oocyte complexes (46%) or from the cleaved ones (63%), hatching rate (69%), and the total number of blastomeres (187). In experiment 2, there was no difference of MII rate between slaughterhouse oocytes cultured for 18 or 22 hours, whereas the MII rate increased significantly ($P < 0.05$) between 18 and 22 hours for LOPU oocytes in the simplified medium. Moreover, slaughterhouse oocytes cultured in simplified medium matured significantly faster than LOPU oocytes at 18 and 22 hours ($P < 0.05$). In experiment 3, cleavage rate was significantly greater ($P < 0.001$) in all four groups of embryos produced by PA than IVF. Interestingly, PA reached similar rates for slaughterhouse oocytes cultured in both media, but improved ($P < 0.05$) the cleavage rate of LOPU oocytes. Slaughterhouse oocytes had acceptable cleavage rate after IVF (~67%), whereas LOPU oocytes displayed a lower one (~38%), in contrast to cleavage after PA. The percentage of blastocysts in relation to cleaved embryos was not affected by the origin of the oocytes ($P > 0.05$). Therefore, slaughterhouse oocytes developed a greater proportion of blastocysts than LOPU ones, expressed as the percentage of total cumulus oocyte complexes entering to IVM. Vitrified-thawed blastocysts presented similar survival and hatching rates between the oocyte origin, media, or method of activation. In conclusion, slaughterhouse and LOPU derived oocytes may have different IVM kinetics and require different IVM and IVF

* Corresponding author. Tel.: +33 608 279 759; fax: +33 2 47 42 77 43.
E-mail address: joannavet@gmail.com (J.M.G. Souza-Fabjan).

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conditions. Although the IVM and IVF systems still need improvements to enhance embryo yield, the *in vitro* development step is able to generate good quality embryos from LOPU-derived oocytes.

1. Introduction

Goats are well adapted to many different environments, and they are very versatile as producers of food and raw matter such as milk, meat and skin. Efficient reproductive biotechnologies are essential to sustain worldwide production. Over the recent years, researchers have been trying to determine which conditions are needed during IVM, IVF, and *in vitro* development (IVD) processes to enhance embryo production to optimize the technique, allowing increasing its adoption in near future. Regarding maturation process in mammals, primary oocytes enter meiosis in perinatal life, progress to the diplotene stage of prophase I (germinal vesicle stage; GV), and remain arrested, eventually until shortly before the time of ovulation. Resumption of meiosis is mediated *in vivo* under the influence of hormonal stimuli and *in vitro* by releasing oocytes from the follicular meiotic inhibiting environment, and further culturing them in suitable conditions. However, it was well demonstrated that IVM oocytes are compromised in their developmental capacity compared with those matured *in vivo* [1,2]. The lower potential of IVM oocytes is probably related to the heterogeneity of the oocytes obtained, in terms of differentiation status, and to inappropriate conditions used in IVM [3]. This results in a relatively low rate of oocytes finally reaching the blastocyst stage, which is one of the main limitations of IVP from immature oocytes in mammals. Therefore, remains a challenge to enhance IVM rates to obtain good quality IVP embryos.

In most of the studies, the basic medium is supplemented with hormones and different concentrations of serum [3,4]. However, all complex supplements such as fetal calf serum (FCS), estrus goat and/or sheep serum or follicular fluid lead to a lack of reproducibility and risk of pathogen contamination. For these reasons, there is a trend to use defined or at least semi-defined maturation media, but this information for goat oocyte is still incipient. To make IVM simpler and more repeatable, we proposed a simplified maturation medium, tissue culture medium (TCM 199), supplemented with 10 ng/mL epidermal growth factor (EGF) and 100 μM cysteamine, and obtained good results in embryo development using slaughterhouse oocytes [5]. Currently, oocytes used for IVP are collected from slaughterhouse ovaries or by LOPU from live animals. Slaughterhouse ovaries provide a cheap source of large number of oocytes usually from unknown females, which are helpful for research and improvement of IVP conditions. However, the use of IVP for genetic improvement or diffusion requires collecting oocytes by repeated LOPU from given females with high economic or genetic merit. Therefore, both sources are equally important to be studied. Earlier trials performed in our laboratory suggest that oocyte requirements during maturation may differ according to their origin, i.e., slaughterhouse or LOPU derived and, thus, the same maturation media could have different efficiencies on both sources [6].

The time required for IVM varies among different species. Earlier studies reported that IVM of goat oocytes should last at least 27 hours [7] or even 32 hours [8], when comparing from 0 to 36 hours. The authors justified that this long time was related to the origin of oocyte (slaughterhouse ovaries that were not stimulated by gonadotropins). However, in the last decade, we have been using 22 to 24 hours of IVM for slaughterhouse goat oocytes with good results in terms of maturation rates and blastocyst production [3,5,9]. Interestingly, a recent study reported longer IVM for slaughterhouse oocytes (24–27 hours) than for LOPU (18–21 hours), probably because the latter were collected from stimulated goats, and already primed for maturation [10]. However, no studies were performed to evaluate the kinetics of maturation of goat LOPU oocytes or, even further, a direct comparison between the IVM kinetics of both sources of oocytes.

Besides the enhanced number of blastocysts at the end of the process, another challenge is to make sure that these embryos are of good quality. The best way to assess embryo quality or viability is to check their capacity of establishing pregnancy, and consequently give birth to normal offspring after transfer to synchronized recipient. However, embryo transfers being heavy and costly in domestic species, some other reliable indicators of embryo viability may be used: the evaluation of the level of expression of specific gene sets, the cell number and allocation (inner cell mass and trophectoderm), kinetic of development, and the resistance to cryopreservation [11]. Vitrification has proven to be as effective as slow cooling methods to cryopreserve mammalian embryos, it was tested in goats with good results [12,13], and can be used for goat blastocysts quality evaluation by postthawing *in vitro* survival score.

Most studies carried out to identify the factors influencing IVM, and subsequent embryo development of goat oocytes were performed using slaughterhouse ovaries. Therefore the aims of this study were to examine the (1) effect of IVM medium composition on maturation rate, fertilization, and embryo development for slaughterhouse oocytes; (2) IVM kinetics of slaughterhouse and LOPU-derived oocytes when submitted to different maturation media; and (3) the developmental competence of slaughterhouse and LOPU-derived oocytes when submitted to different maturation media, and submitted to IVF or parthenogenetic activation (PA).

2. Material and methods

Except otherwise indicated, chemicals were purchased from Sigma Chemical Co. (Saint Louis, MO, USA).

All the experiments were conducted at the Experimental Unit Unité expérimentale de Physiologie Animale de l'Orfrasière in Nouzilly (France, latitude 47°22'N, longitude

00°41'E). The procedures were approved by the local ethic committee. Three experiments were performed to examine the impact of different aspects of IVM on maturation, fertilization, embryo development, and quality of goat oocytes obtained by LOPU or slaughterhouse ovaries.

2.1. Experiment 1

2.1.1. Experimental design

The objective of this experiment was to determine the effect of IVM medium composition on the maturation, fertilization, cleavage, and embryo development rates. In total, 846 cumulus oocyte complexes (COCs) obtained by slaughterhouse ovaries were analyzed in four replicates.

2.1.2. Aspiration of oocytes

During the nonbreeding season (March and April), ovaries from goats, regardless the stage of estrous cycle, were collected from a local slaughterhouse and transported to the laboratory in a thermos box containing saline solution (0.9% NaCl) at 30 °C within 4 hours after collection. Ovaries were washed in prewarmed fresh saline (30 °C), and oocytes were aspirated from all visible follicles between 2 and 6 mm in diameter with a 18-ga short needle connected to a Falcon tube under controlled vacuum (30 mm Hg). The collection tube was previously filled with 3 to 5 mL of HEPES-buffered TCM 199 supplemented with 10 IU/mL heparin (Choay; Glaxo Wellcome Production, Notre Dame de Bondeville, France), 4 μg/mL gentamicin, and 1 mg/mL BSA (A 9647).

2.1.3. IVM of oocytes

Cumulus oocyte complexes were isolated under a stereo zoom microscope (Nikon Corporation, Japan), and graded as grade 1, 2, or 3. Only good quality (grades 1 and 2), i.e., surrounded by at least one complete layer of unexpanded cumulus cells and finely granulated oocyte cytoplasm were considered acceptable and used in IVM [14], whereas grade 3 (incomplete cellular investment or heterogeneous oocyte cytoplasm) oocytes were discarded. The COCs were washed four times, and transferred to four-well petri dishes (Nunc, Roskilde, Denmark) containing 45 to 50 oocytes in 500 μL of maturation medium. The maturation medium consisted of TCM 199 supplemented either with: (1) 10 ng/mL EGF and 100 μM cysteamine (EGF medium; simplified); (2) 10% FCS, 100 μM cysteamine, and 50 ng/mL ovine FSH (FCS medium; complex); or (3) 10 ng/mL EGF, 5 UI/mL hCG, 10 UI/mL eCG, 19 ng/mL Insulin-like Growth Factor-1 (IGF-1), 2.2 ng/mL Fibroblast Growth Factors (FGF), 5 μg/mL insulin, 5 μg/mL transferine, 5 ng/mL selenium, 90 μg/mL L-cysteine, 0.1 mM β-mercaptoethanol, 75 μg/mL vitamin C, 720 μg/mL glycine, 0.1 mg/mL glutamine, and 110 μg/mL pyruvate (MIX, semi-defined). The COCs were incubated for 22 hours at 38.8 °C in a humidified atmosphere of 5% CO_2 in air [1].

2.1.4. Determination of stage of nuclear maturation

After 22 hours of IVM, part of the oocytes (approximately 25%) from each maturation treatment was placed into 15 mL Falcon tubes containing 2 mL of TCM 199 medium and BSA (2 μL/mL), and vortexed for 2 minutes (medium speed) to remove cumulus oophorus. These oocytes were recovered in 35 mm petri plates, washed, transferred within a 10-μL droplet onto a grease-free slide, and dried. They were then fixed in ethanol 100% for at least 3 hours. After dying, oocytes were stained in 10 μg/mL Hoechst 33342 fluorochrome (stains all nuclei) in Vectashield mounting medium (Vector Labs, Burlingame, CA, USA), and overlaid with a cover slip sealed with nail varnish. Slides were stored at 4 °C until they were examined using epifluorescent microscopy to evaluate the meiosis nuclear stage and the presence or absence of the first polar body.

2.1.5. Sperm preparation and IVF of oocytes

Two straws per replicate of semen from the same ejaculate and/or buck were used throughout experiments. Motile sperm from frozen/thawed semen were separated by centrifugation (15 minutes at 700× *g*) on 2 mL of Percoll (Pharmacia, Uppsala, Sweden) discontinuous density gradient (45%/90%). Viable sperm were diluted in the appropriate volume of fertilization medium, to achieve a final concentration of 2.0×10^6 sperm/mL (Day of IVF = Day 0).

The matured COCs were transferred into plates containing washing medium. The washing and fertilization medium used were synthetic oviduct fluid (SOF) medium (pH = 7.3; 280 mOsm), which contained 10% of heat-inactivated estrus sheep serum, 5 μg/mL heparin (Calbiochem 375 095), and 4 μg/mL gentamicin. Groups of 45 to 50 oocytes were transferred into four-well petri dishes containing 450 μL of fertilization medium and 50 μL of sperm suspension were added to each well. Sperm and oocytes were coincubated for 18 hours at 38.8 °C in a humidified atmosphere of 5% CO_2 in air [9].

2.1.6. Fertilization rate

At the end of IVF, all COCs were denuded by vortex as described earlier. Part of the oocytes (approximately 25% of the initial number) from each maturation treatment were fixed and stained with Hoechst, as previously detailed (Section 2.1.4). Sperm penetration and pronuclear formation were assessed under an epifluorescence microscope. The presence of three or more pronuclei or decondensing sperm heads was designated as polyspermia.

2.1.7. IVD of embryos

The other presumptive zygotes (approximately 50% of the initial number) were washed four times in culture medium (SOF supplemented with 3 mg/mL BSA) to remove spermatozoa, and transferred by groups of 25 into four-well petri dishes containing 25 μL drops of culture medium covered with 700 μL of mineral oil. The presumptive zygotes were cultured for 7 days at 38.8 °C in a humidified atmosphere of 5% O_2, 5% CO_2, and 90% N_2. After 48 hours post-insemination (PI), 10% FCS was added to the culture droplets.

Embryos were examined morphologically, and the efficiency of development was evaluated (1) as the percentage of cleaved embryos 2 days PI, and the percentage of blastocysts at 8 days PI, expressed; (2) on the basis of the number of oocytes entering into IVM; or (3) on the basis of the number of cleaved embryos at Day 2. On Day 8, all expanded blastocysts were transferred into washing plates, washed to remove the mineral oil, fixed and stained with Hoechst to count their total cell number. Cell counting was conducted under an epifluorescence microscope.

2.2. Experiment 2

2.2.1. Experimental design

The objective of this experiment was to determine the effect of oocyte origin (slaughterhouse ovaries or LOPU) on the kinetics of maturation when submitted either to simplified (EGF) or semi-defined (MIX) maturation media. Oocytes from both origins were cultured for 18, 22, or 26 hours. The effect of COC morphology on maturation kinetic was also evaluated. No IVF was performed in this experiment. Consequently, this experiment was comparing two types of oocytes (LOPU vs. slaughterhouse), two IVM media (EGF vs. MIX), and three maturation times (18 vs. 22 vs. 26 hours). In total, 545 oocytes were used from slaughterhouse ovaries and 423 from LOPU, in three replicates.

2.2.2. Aspiration of oocytes—slaughterhouse ovaries and LOPU in live goats

In the subsequent months (May and June), the same procedure as in experiment 1 was adopted to recover the oocytes from slaughterhouse ovaries. Laparoscopic ovum pick up sessions were organized on the same day of slaughterhouse-oocyte recovery, and conducted in 32 pluriparous Saanen and Alpine goats (alternating 16 goats per week). The animals were housed in free stalls, and provided with food and water *ad libitum*. Females received intravaginal sponges impregnated with 45 mg Fluorogestone Acetate, (Chronogest CR; Intervet Schering-Plough Animal Health, Angers, France) for 11 days and a prostaglandin intramuscular injection (Cloprostenol, 50 μg; Intervet Schering-Plough Animal Health) that was administered 8 days after the Fluorogestone Acetate sponge insertion. At the same moment, they were stimulated with a total of 16 mg FSH (Stimufol, highly purified porcine FSH, kindly provided by Prof. J-F Beckers, Liège, Belgium), which was given as twice-daily intramuscular injections in five decreasing dose regimen (4, 4, 3, 3, and 2 mg). Fluorogestone Acetate sponges were removed at the moment of LOPU.

The goats were deprived of feed and water for 24 hours before laparoscopy. Animal suffering was avoided by proceeding under general anesthesia with xylazine (0.5 mg per 10 kg of Kensol 2%; Konig, France) and ketamine (25 mg per 10 kg of Ketalar; Parke-Davis). The animal was placed in an inverted position on a cradle at a 45° angle. The aspiration was performed by the use of a 5 mm laparoscope attached to a video system. The endoscope was inserted into the abdominal cavity through a trocar, cranial to the udder and on the left of midline. A second trocar was inserted into the right side (opposite from the first one) of the abdomen for passing a nontraumatic grasping forceps. A third trocar was inserted in the midline for passing the oocyte retrieval needle. The ovary was held by the grasper, and all ovarian follicles greater than 2 mm were individually aspirated using a 18-ga needle (Cook Ireland Ltda, Limerick, Ireland) connected to an aspiration system. The vacuum pressure was set at 50 mm Hg. The collection tube was previously filled with 3 to 5 mL of HEPES-buffered TCM 199 supplemented with 10 IU/mL heparin (Choay, Glaxo Wellcome Production), 4 μg/mL gentamicin, and 1 mg/mL BSA (A 9647). After aspiration, each ovary was gently washed with a heparinized saline solution (25 IU/mL) at 37 °C for the prevention of possible adhesions. Finally, the trocar orifices were treated with a local antibiotic healing solution.

2.2.3. Assessment of COC quality and IVM of oocytes

Part of the oocytes from the first goat (around 9 AM) submitted to LOPU was retrieved, washed and lasted in the washing medium until the last goat was aspirated (16th, around 12 PM), which also had part of her oocytes separated and washed. These oocytes were denuded, fixed, and stained with Hoechst. The same process was performed with a random ovary aspirated (slaughterhouse ovaries collected around 10 AM, and aspiration performed at 2 PM). The stages of oocyte nuclei upon aspiration (immature oocytes) were determined. By observing nuclear stages, the nuclei were classified into GV, GV breakdown (GVBD), or intermediary stages (metaphase, anaphase, telophase I).

Just after the aspiration of the second and subsequent goats, the collection tubes were transported to the culture room, where the oocytes were retrieved, selected, and transferred to the washing plate. After aspirating approximately half of the goats of the day (the eighth doe, around 10:30 AM), the first LOPU group was pooled and transferred to the maturation media (interval from the first to the eight goat lasted maximum 2 hours, approximately from 8 to 10 AM). The aspiration continued for the ninth to 16th goat, and the second LOPU group was formed (maximum 2 hours between the ninth to 16th doe, approximately from 10 to 12 AM). All good (grade 1 and 2) and bad (grade 3, denuded) quality COCs were used, but grade 3 oocytes were separated in a different group for each origin (LOPU or slaughterhouse). The oocytes were washed four times, and finally transferred by groups of 45 to 50 oocytes into wells containing 500 μL of maturation medium. The maturation media used were either EGF or MIX, both described in experiment 1. IVM lasted 18, 22, or 26 hours, with one-third of the oocytes removed at each time point, each maturation medium and both quality groups (grade 1 and 2 or grade 3). Time points occurred at different moments for the three groups of oocyte origins (first LOPU, second LOPU, and slaughterhouse oocytes). At each moment, one-third of the oocytes in each well (three different times of maturation, two different maturation media, and two different oocytes qualities) was recovered, washed, denuded, fixed, and stained with Hoechst, as previously detailed in experiment 1. At the end, the data from both LOPU groups were pooled and compared with slaughterhouse oocytes.

2.3. Experiment 3

2.3.1. Experimental design

The objective of this experiment was to determine the differences between the oocyte origin (slaughterhouse ovaries and LOPU) when submitted to simplified (EGF) or semi-defined (MIX) maturation media and then to either IVF or PA. Embryo quality was evaluated through vitrification and warming analyses. Consequently, this experiment was comparing two types of oocytes (LOPU vs. slaughterhouse), two IVM media (EGF vs. MIX), and two methods of activation (IVF vs. PA). A total of 1043 oocytes were used

from slaughterhouse ovaries and 570 from LOPU, in four replicates.

2.3.2. Aspiration of oocytes

In the subsequent months (July to November), the same procedures as in experiment 2 were adopted to recover the oocytes from slaughterhouse ovaries and LOPU. In the current experiment, other 32 pluriparous Saanen and Alpine goats were selected. The same stimulatory treatment and aspiration procedure were employed for 16 animals per week, alternatively.

2.3.3. Assessment of COC quality and IVM of oocytes

The COCs from both origins were isolated and graded. The oocytes were washed four times and placed in wells containing 45 to 50 oocytes in 500 μL of maturation medium. The maturation media used were either EGF or MIX, both previously described in experiment 1. Considering the quality of the oocytes, grade 1 and 2 (G1/2) oocytes from slaughterhouse were pooled as a control, whereas grades 1, 2, and 3 (G1/2/3) were pooled in other groups. IVM lasted for 22 hours on average in all treatments. The six treatments for IVF were LOPU EGF, G1/2/3; LOPU MIX, G1/2/3; slaughterhouse EGF, G1/2/3; slaughterhouse MIX, G1/2/3; slaughterhouse EGF, G1/2; and slaughterhouse MIX, G1/2. For oocytes submitted to PA, all three grades were pooled and were cultured in IVM for 24 hours, the four treatments were LOPU EGF, G1/2/3; LOPU MIX, G1/2/3; slaughterhouse EGF, G1/2/3; and slaughterhouse MIX, G1/2/3.

2.3.4. IVF of oocytes

After 22 hours of maturation, oocytes from each of the six treatments were washed and transferred into IVF medium. Semen preparation, fertilization medium, and IVF followed the same protocol as that used in experiment 1.

2.3.5. Parthenogenetic activation

After 24 hours of maturation, oocytes from each of the four treatments were submitted to PA. Briefly, all COCs were denuded and transferred to four-well plates, with each well containing 500 μL of medium. After vortexing for denuding, the oocytes were washed two times in TCM 199 containing 5% FCS and 1 μL/mL gentamicin. Then, oocytes were activated for 5 minutes in the same medium supplemented with 2 μL/mL ionomycin, and washed again three times. Finally, they were transferred into the same medium containing 5 μL/mL 6-dimethylaminopurine (6-DMAP) during 4 hours to inactivate M-phase promoting factor activity. After the activation process, oocytes were washed three more times and washed a last time in IVD medium before placing them to IVD medium.

2.3.6. IVD of presumptive zygotes

At the end of IVF, all presumptive zygotes were denuded by vortex, washed four times in culture medium, and cultured for 8 days in microdroplets for development. All the procedure and media are detailed in experiment 1. Embryos were examined morphologically for cleavage and embryo development rates as earlier described in experiment 1 (Section 2.1.7).

2.3.7. Vitrification and warming

The ability of the blastocysts to survive cryopreservation was used as a tool to assess their quality [15]. On Day 8 PI, all blastocysts were transferred into washing plates, washed to remove the mineral oil, and vitrified as previously described [16]. Embryos of the same stage of development were vitrified together in the same straw (young and expanded or hatched blastocysts). Briefly, embryos were kept at room temperature for 5 minutes in PBS supplemented with 20% new-born calf serum (NBCS). Blastocysts were then vitrified in three steps at room temperature as follows: 10% glycerol for 5 minutes, 10% glycerol and 20% ethylene glycol for 5 minutes, and finally 25% glycerol and 25% ethylene glycol for 30 seconds in PBS–NBCS supplemented with 0.4 M sucrose. During the last step, embryos were quickly aspirated into the center part of a 0.25 mL plastic straw (IMV, L'Aigle, France) within 20 to 30 μL of vitrification solution. Embryos were separated by two air bubbles from two surrounding segments of PBS–NBCS containing 0.8 M galactose (about 90 μL each). The straws were sealed with PVA, and immediately plunged directly into liquid nitrogen.

All straws remained from 7 to 15 days in the liquid nitrogen before warming. For warming, straws were held for 5 seconds in air followed by 15 seconds in a 22 °C water bath. The straw content was expelled into an empty petri dish, where embryos were kept for 5 minutes, and were stirred gently to facilitate the mixture of the two solutions. Afterward, the embryos were gently placed into another plate containing PBS–NBCS, and left for 5 minutes at room temperature. They were subsequently washed in the same medium at 39 °C, and placed in culture for 72 hours (SOF medium containing 10% FCS, 500 μL per well in a four-well plate, embryos from one straw in each well). Survival rates were evaluated with morphologic criteria, on the basis of the integrity of the embryo membranes and the zona pellucida (with the exception of hatched blastocysts), and re-expansion of the blastocoel. Furthermore, the percentages of embryos that resumed their development and reached a more advanced developmental stage after culture were recorded (development rate). The embryos were evaluated at 6, 24, 48, and 72 hours [17].

2.4. Statistical analysis

One-way ANOVA was performed for the comparison of the number of cells among different treatments in experiment 1. Differences between replicates were tested by chi-square test within each treatment and differences among treatments at cleavage rate, embryo development, and survival rates after vitrification and/or warming. A value of $P < 0.05$ was considered to be statistically significant. Statistical analyses were performed using GraphPad Prism 5.0a software.

3. Results

3.1. Experiment 1

A more important cumulus expansion was subjectively after IVM in complex and semi-defined in comparison with simplified medium (Fig. 1). However, no significant difference

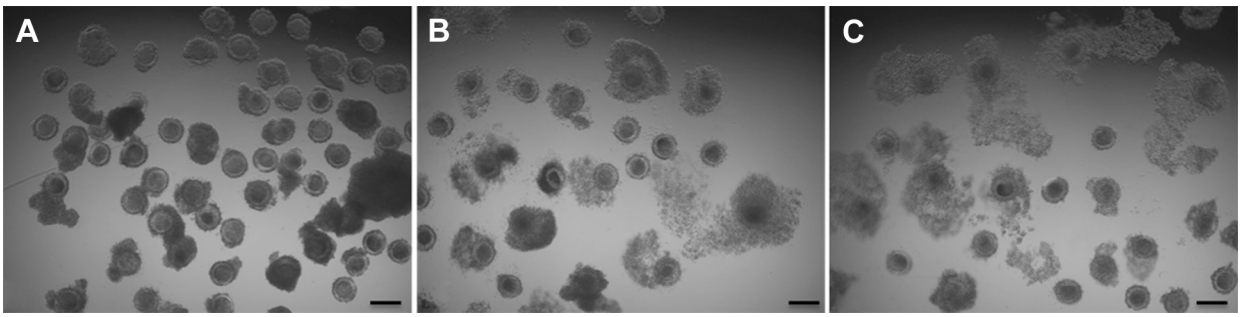

Fig. 1. Cumulus cell expansion of goat COC after 22 hours of IVM in medium consisting of TCM 199 supplemented with: (A) 10 ng/mL epidermal growth factor (EGF) and 100 μM cysteamine; (B) 10% FCS, 100 μM cysteamine, and 50 ng/mL oFSH; or (C) MIX, 10 ng/mL EGF, 5 UI/mL hCG, 10 UI/mL eCG, 19 ng/mL IGF-1, 2.2 ng/mL FGF, 5 μg/mL insulin, 5 μg/mL transferine, 5 ng/mL selenium, 90 μg/mL L-cysteine, 0.1 mM β-mercaptoethanol, 75 μg/mL vitamin C, 720 μg/mL glycine, 0.1 mg/mL glutamine, and 110 μg/mL pyruvate. The bar represents 500 μm. COC, cumulus complex oocyte; oFSH, ovine FSH; TCM, tissue culture medium.

was detected on maturation rate among the three treatments (Table 1). When the data were pooled regardless of the treatment, average maturation was 88 ± 2.0%, intermediate stages 8 ± 1.4% and GV rate only 4 ± 1.0%. Likewise, the different maturation media promoted similar rates of fertilization, averaging 72 ± 2.4% (Table 2). High rates of polyspermic penetration (Fig. 2) were verified in the present study, averaging 34 ± 2.4% and monospermic penetration rate was 38 ± 3.2% (not shown). Embryo development is shown in Table 3. The maturation medium did not influence the cleavage (72 ± 3.0% on average), the blastocyst rate from the initial number of COCs entering to IVM (46 ± 1.1%) or from the cleaved ones (63 ± 2.1%), the hatching rate (69 ± 3.3%), and the total number of cells per expanded blastocyst (187 ± 26.1).

3.2. Experiment 2

Although not significantly different, more slaughterhouse oocytes were observed at a meiotic resumption stage at collection (GVBD, 19/26; 73%), than immature oocytes from LOPU, either from the first (7/13; 54%) or last goat (6/11; 55%). Consequently, the GV stage was higher in LOPU, for the first (6/13; 46%) or last goat (5/11; 45%) than in slaughterhouse oocytes (7/26; 27%).

Laparoscopic ovum pick up and slaughterhouse oocytes may have different maturation kinetics, because significant effect of culture time on nuclear maturation of good quality oocytes (grade 1 and 2) were observed according to oocyte origin. At 18 hours of maturation, similar results were obtained in comparison with 22 hours for slaughterhouse oocytes. Laparoscopic ovum pick up oocytes matured in EGF showed a higher rate of maturation ($P < 0.05$), when cultured for 22 than 18 hours. Moreover, slaughterhouse oocytes cultured in EGF matured significantly faster than LOPU oocytes (18 and 22 hours, $P < 0.05$), whereas no difference was observed when oocytes from both origins were matured in MIX. Although at 22 and 26 hours of maturation slaughterhouse oocytes showed similar rates when comparing both maturation media, at 18 hours, EGF showed greater ($P < 0.05$) maturation rate than MIX (Table 4).

Interesting results were obtained when comparing good (grade 1 and 2) with bad quality (grade 3) oocytes. It is noteworthy that regarding oocyte quality, no difference was detected in maturation rate of LOPU oocytes at 18, 22, and 26 hours, respectively, between EGF (50%, 54%, and 44%) or MIX (62%, 53%, and 64%). Conversely, significant differences were found in slaughterhouse oocytes between good and bad quality oocytes, respectively, at 18 hours (87% vs. 67%; EGF), 22 hours (90% vs. 66%; EGF), and 26 hours (90% vs. 70%; MIX). At the other moments, the low quality of oocytes did not adversely influence maturation rates, which reached 80% (26 hours; EGF), 47% (18 hours; MIX), and 88% (22 hours; MIX). Only slaughterhouse oocytes cultured in EGF for 22 hours led to higher ($P < 0.05$) degenerated oocyte rates for bad quality in relation to good ones (17% vs. 2%), whereas all the other 11 groups had similar degenerated rates. No difference was observed in GV rate in any group. Bad quality oocytes from both origins demonstrated similar maturation rates, whereas slaughterhouse had higher ($P < 0.05$) maturation rate than LOPU oocytes, respectively, at 26 hours for EGF (80% vs. 44%) or 22 hours for MIX (88% vs. 53%). Interestingly, for slaughterhouse oocytes cultured for 22 hours, MIX promoted higher ($P < 0.05$) maturation rate than EGF.

Table 1
Effect of different maturation media on the nuclear maturation of slaughterhouse goat oocytes submitted to IVM for 22 hours (mean ± SEM).

Treatment	n	GV (%)	Intermediate[a] (%)	MII (%)
EGF[b]	66	3 (4 ± 2.0)[e]	7 (11 ± 3.6)[e]	56 (85 ± 4.7)[e]
FCS[c]	74	2 (3 ± 2.1)[e]	4 (5 ± 2.1)[e]	68 (92 ± 3.6)[e]
MIX[d]	70	3 (4 ± 1.3)[e]	6 (9 ± 1.5)[e]	61 (87 ± 2.2)[e]

n represents the number of IVM oocytes evaluated.
[e] Within a column, values without a common alphabet differ significantly by chi-square ($P < 0.05$).
Four replicates were used in this experiment.
Abbreviations: GV, germinal vesicle; MII, metaphase II; SEM, standard error of the mean.
[a] Intermediary (metaphase I, anaphase I, and telophase I).
[b] EGF: 10 ng/mL epidermal growth factor and 100 μM cysteamine.
[c] FCS: 10% fetal calf serum, 100 μM cysteamine, and 50 ng/mL oFSH.
[d] MIX: 10 ng/mL EGF, 5 UI/mL hCG, 10 UI/mL eCG, 19 ng/mL IGF-1, 2.2 ng/mL FGF, 5 μg/mL insulin, 5 μg/mL transferine, 5 ng/mL selenium, 90 μg/mL L-cysteine, 0.1 mM β-mercaptoethanol, 75 μg/mL vitamin C, 720 μg/mL glycine, 0.1 mg/mL glutamine, and 110 μg/mL pyruvate.

3.3. Experiment 3

Cleavage and development rates to the blastocyst stage are shown in Table 5. Cleavage rate was significantly higher ($P < 0.001$) in all four groups of embryos produced by PA compared with IVF. Interestingly, PA induced similar

Table 2
Effect of different maturation media on the IVF of goat slaughterhouse oocytes (mean ± SEM).

Treatment	n	Monospermic penetration (%)	Polyspermic penetration (%)	Unfertilized (%)	Fertilized[a] (%)
EGF[b]	76	28 (37 ± 7.1)[e]	28 (37 ± 5.9)[e]	20 (26 ± 4.0)[e]	56 (74 ± 3.1)[e]
FCS[c]	78	30 (39 ± 4.1)[e]	22 (28 ± 1.5)[e]	26 (33 ± 4.3)[e]	52 (67 ± 4.3)[e]
MIX[d]	78	29 (37 ± 6.5)[e]	29 (37 ± 3.6)[e]	20 (26 ± 5.2)[e]	58 (74 ± 5.2)[e]

n represents the number of presumptive zygotes evaluated.
[e] Within a column, values with different alphabets differ significantly by chi-square ($P < 0.05$).
Four replicates were used in this experiment.
Abbreviation: SEM, standard error of the mean.
[a] Total % of monospermic and polyspermic zygotes.
[b] EGF: 10 ng/mL epidermal growth factor and 100 μM cysteamine.
[c] FCS: 10% fetal calf serum, 100 μM cysteamine, and 50 ng/mL oFSH.
[d] MIX: 10 ng/mL EGF, 5 UI/mL hCG, 10 UI/mL eCG, 19 ng/mL IGF-1, 2.2 ng/mL FGF, 5 μg/mL insulin, 5 μg/mL transferine, 5 ng/mL selenium, 90 μg/mL L-cysteine, 0.1 mM β-mercaptoethanol, 75 μg/mL vitamin C, 720 μg/mL glycine, 0.1 mg/mL glutamine, and 110 μg/mL pyruvate.

cleavage rates for slaughterhouse oocytes cultured in both media, but higher cleavage rate was observed after PA, when LOPU oocytes were matured in MIX in comparison with EGF. This improvement was not observed after IVF. As in experiment 2, LOPU and slaughterhouse oocytes showed differences, whereas slaughterhouse oocytes had acceptable cleavage rates after IVF (~67%), the cleavage of LOPU oocytes was low (~38%), whatever the maturation medium used. In addition, slaughterhouse oocytes had higher cleavage rates than LOPU ones in both media after IVF, but not after PA. The percentage of blastocysts in relation to cleaved embryos was not affected either by the oocyte origin, maturation medium, or method of embryo production. However, slaughterhouse oocytes developed to blastocysts in a higher proportion than LOPU ones, when expressed as percentage of the total COCs entering in IVM, reflecting the cleavage rate (Table 5). Our control groups using only grade 1 and 2 slaughterhouse oocytes, submitted to IVF only, showed no differences when compared with equivalent groups formed by grades 1, 2, and 3 oocytes (ranging 0.93–1.09, highlighting its statistical and numerical similarities). Regarding our control groups, similar results were obtained between EGF and MIX, respectively, for cleavage (69 ± 2.4% [129/187] and 71 ± 1.4% [130/183]), percentage of blastocysts from cleaved embryos (64 ± 1.6% [83/129] and 63 ± 6.3% [82/130]), percentage of blastocysts from initial COC (44 ± 2.1% and 45 ± 4.2%) and hatching rate (65 ± 3.9% [54/83] and 66 ± 5.4% [54/82]).

Blastocyst re-expansion and hatching rates after vitrification, warming, and 72 hours of IVC are summarized in Table 6. Although differences were detected between expanded and hatched embryos from the same groups, similar results were obtained in survival and hatching rates between the oocyte origin, maturation medium, or method of embryo production (Table 6). Our control groups had similar survival and hatching rates when grade 3 oocytes were mixed (data not shown).

4. Discussion

The main objectives of the present study were to compare the behavior of goat oocytes recovered from slaughterhouse ovaries or by LOPU in live goats submitted to different IVM treatments. Five main conclusions can be drawn from our study. First, it is possible to achieve high maturation, fertilization, cleavage rates, and embryo development when using simplified and semi-defined maturation media in slaughterhouse oocytes. Second, slaughterhouse and LOPU-derived oocytes have different IVM kinetics, with the latter requiring more time when

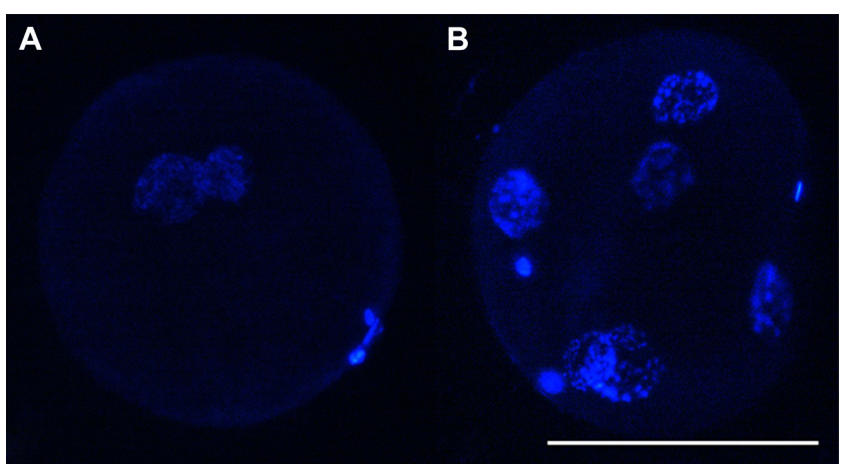

Fig. 2. IVF of goat oocyte. (A) Monospermic penetration, with two pronuclei; and (B) polyspermic penetration, with six visible pronuclei. The bar represents 100 μm.

Table 3
Effect of different maturation media on developmental competence of goat embryos produced from slaughterhouse oocytes. Percentages of cleavage, blastocysts (Bl), and hatched embryos in relation to the total blastocysts (Hbl/totBl) and blastocysts cell counts (mean ± SEM).

Treatment	n	Cleavage (%)	Bl/COC (%)	Bl/cleaved (%)	Hbl/totBl (%)	Total cells (n)[a]
EGF[b]	141	77 ± 3.4[e]	46 ± 0.5[e]	60 ± 2.2[e]	72 ± 6.4[e]	192 ± 13.7 (18)[e]
FCS[c]	132	72 ± 4.8[e]	45 ± 2.9[e]	63 ± 3.8[e]	67 ± 5.1[e]	179 ± 13.9 (19)[e]
MIX[d]	131	66 ± 4.8[e]	45 ± 1.4[e]	68 ± 3.0[e]	68 ± 4.6[e]	189 ± 11.4 (19)[e]

n represents the number of IVM oocytes submitted to IVF and IVD.
[e] Within a column, values with different alphabets differ significantly ($P < 0.05$) by chi-square.
Four replicates were used in this experiment.
Abbreviations: COC, cumulus oocyte complex; IVD, in vitro developed; oFSH, ovine FSH; SEM, standard error of the mean.
[a] ANOVA test ($P > 0.05$).
[b] EGF: 10 ng/mL epidermal growth factor and 100 μM cysteamine.
[c] FCS: 10% fetal calf serum, 100 μM cysteamine, and 50 ng/mL oFSH.
[d] MIX: 10 ng/mL EGF, 5 UI/mL hCG, 10 UI/mL eCG, 19 ng/mL IGF-1, 2.2 ng/mL FGF, 5 μg/mL insulin, 5 μg/mL transferine, 5 ng/mL selenium, 90 μg/mL L-cysteine, 0.1 mM β-mercaptoethanol, 75 μg/mL vitamin C, 720 μg/mL glycine, 0.1 mg/mL glutamine, and 110 μg/mL pyruvate.

submitted to EGF medium. Third, LOPU oocytes had lower cleavage potential than slaughterhouse ones after IVF, not after PA. Fourth, LOPU and slaughterhouse oocytes have similar developmental competence, providing similar blastocyst rates after PA. Fifth, the embryos produced by IVF or PA have similar survival rates, demonstrating that although the IVM and IVF systems still need improvements to enhance embryo yield, the IVD step is able to generate good quality embryos from LOPU-derived oocytes.

The use of complex, simplified (EGF), or semi-defined (MIX) maturation media promoted similar maturation, fertilization and cleavage rate, embryo development, and quality in slaughterhouse-derived oocytes. The average maturation rate (88%) obtained is considerably high, because lower rates are often reported in the literature, even when using serum (66%) [2]. As a measure of IVF success, usually only cleavage rate is evaluated. However, in some cases, the sperm penetration rate is different from cleavage as for example in comparison between young and adult females [18]. The present work demonstrated that sperm penetration (72%) was similar to cleavage rate (72%) in all three IVM media tested. It is noteworthy that high rates of polyspermic penetration were verified in the present study (~34% in average regardless of the treatment). Even higher polyspermic rate (45%) was reported in prepubertal goats [19], implying that polyspermia in goats is a recurrent event, at least *in vitro*.

Embryo quality was evaluated on the basis of the number of cells in expanded blastocysts at Day 8, which did not differ significantly among treatments. Our finding corroborated an earlier report in ovine [20] but was contrary to those previously reported in cattle, when the IVM medium supplemented with serum resulted in blastocysts with a larger number of cells [21]. We obtained an average of 187 cells, greater than 120 [22] or 130 [23] and lesser than 243 cells, but counted on Day 9 [24], all reports in goats. It was previously described that morphologic appearance did not necessarily indicate the true developmental status of the goat embryo [22], however, all expanded blastocysts selected on morphology in the

Table 4
Effect of culture time on nuclear maturation of good quality goat (grade 1 and 2) oocytes obtained after LOPU or slaughterhouse ovaries, and submitted to different maturation media (mean ± SEM).

Oocyte origin	IVM media	Culture time (h)	n	Degenerated (%)	GV (%)	Intermediate[a] (%)	MII (%)
LOPU	EGF[b]	18	57	3 (5 ± 2.9)[d,x,f]	12 (21 ± 7.4)[d,x,f]	12 (21 ± 3.1)[d,x,f]	30 (53 ± 7.2)[d,x,f]
		22	50	1 (2 ± 2.1)[d,x,f]	7 (14 ± 3.9)[d,x,f]	6 (12 ± 7.2)[d,x,f]	36 (72 ± 1.8)[e,x,f]
		26	52	1 (2 ± 2.1)[d,x,f]	8 (15 ± 4.4)[d,x,f]	9 (17 ± 2.3)[d,x,f]	34 (65 ± 4.6)[d,e,x,f]
	MIX[c]	18	57	6 (11 ± 5.5)[d,x,f]	6 (11 ± 2.7)[d,x,f]	10 (18 ± 2.0)[d,x,f]	35 (61 ± 6.3)[d,x,f]
		22	50	2 (4 ± 1.9)[d,x,f]	1 (2 ± 1.6)[d,y,f]	14 (28 ± 6.4)[d,y,f]	33 (66 ± 8.7)[d,x,f]
		26	50	1 (2 ± 1.2)[d,x,f]	1 (2 ± 1.2)[d,y,f]	10 (20 ± 6.0)[d,x,f]	38 (76 ± 6.4)[d,x,f]
Slaughterhouse	EGF[b]	18	46	1 (2 ± 4.2)[d,x,f]	0 (0 ± 0.0)[d,x,g]	5 (11 ± 7.4)[d,x,f]	40 (87 ± 3.2)[d,x,g]
		22	48	1 (2 ± 1.8)[d,x,f]	1 (2 ± 1.8)[d,x,g]	3 (6 ± 3.2)[d,x,f]	43 (90 ± 0.4)[d,x,g]
		26	56	3 (5 ± 0.7)[d,x,f]	2 (4 ± 2.9)[d,x,g]	7 (12 ± 8.4)[d,x,f]	44 (79 ± 4.8)[d,x,f]
	MIX[c]	18	42	2 (5 ± 2.8)[d,x,f]	1 (2 ± 1.4)[d,x,f]	13 (31 ± 1.4)[d,y,f]	26 (62 ± 2.8)[d,y,f]
		22	54	3 (6 ± 0.4)[d,x,f]	2 (4 ± 2.9)[d,x,f]	6 (11 ± 7.1)[e,x,g]	43 (80 ± 3.7)[d,e,x,f]
		26	51	4 (8 ± 1.8)[d,x,f]	0 (0 ± 0.0)[d,x,f]	1 (2 ± 2.8)[e,y,g]	46 (90 ± 1.0)[e,x,f]

n represents the number of oocytes evaluated.
Within a column, values with different alphabets differ significantly by chi-square ($P < 0.05$).
Three replicates were used in this experiment.
[d,e] differ between the time of maturation (18 vs. 22 vs. 26 hours) within the same medium and origin of the oocyte.
[x,y] differ between the maturation medium (EGF vs. MIX) at the same time of maturation and origin of the oocyte.
[f,g] differ between the origin of oocytes (LOPU vs. slaughterhouse) at the same time of maturation and medium.
Abbreviations: GV, germinal vesicle; LOPU, laparoscopic ovum pick up; MII, metaphase II; SEM, standard error of the mean.
[a] Intermediary (metaphase I, anaphase I, and telophase I).
[b] EGF: TCM 199 supplemented with10 ng/mL epidermal growth factor and 100 μM cysteamine.
[c] MIX: TCM 199 supplemented with 10 ng/mL EGF, 5 UI/mL hCG, 10 UI/mL eCG, 19 ng/mL IGF-1, 2.2 ng/mL FGF, 5 μg/mL insulin, 5 μg/mL transferine, 5 ng/mL selenium, 90 μg/mL L-cysteine, 0.1 mM β-mercaptoethanol, 75 μg/mL vitamin C, 720 μg/mL glycine, 0.1 mg/mL glutamine, and 110 μg/mL pyruvate.

Table 5
Effect of oocyte origin, maturation medium, and activation method to produce goat embryos (IVF or submitted to PA) from a mixed group of oocytes (grade 1, 2, and 3) on embryo developmental competence.

Oocyte origin	IVM system	Method	n	Cleavage (%)	Bl/COC (%)	Bl/cleaved (%)	Hbl/TotBl (%)
LOPU	EGF[a]	IVF	150	39 ± 3.4^{c,x,e}	28 ± 2.4^{c,x,e}	71 ± 4.0^{c}	55 ± 4.8^{c}
		PA	120	83 ± 4.5^{d,x,e}	55 ± 1.9^{d,x,e}	67 ± 5.9^{c}	65 ± 4.7^{c}
	MIX[b]	IVF	151	38 ± 4.0^{c,x,e}	25 ± 4.8^{c,x,e}	64 ± 6.5^{c}	57 ± 4.4^{c}
		PA	149	93 ± 3.5^{d,y,e}	61 ± 5.2^{d,x,e}	65 ± 4.0^{c}	69 ± 2.1^{c}
Slaughterhouse	EGF[a]	IVF	169	68 ± 2.6^{c,x,f}	47 ± 2.4^{c,x,f}	70 ± 4.1^{c}	65 ± 6.5^{c}
		PA	170	89 ± 3.1^{d,x,e}	61 ± 4.9^{d,x,e}	68 ± 4.2^{c}	62 ± 3.3^{c}
	MIX[b]	IVF	170	66 ± 6.6^{c,x,f}	43 ± 3.9^{c,x,f}	65 ± 6.1^{c}	68 ± 3.7^{c}
		PA	164	91 ± 2.3^{d,x,e}	59 ± 5.6^{d,x,e}	64 ± 5.5^{c}	63 ± 5.3^{c}

n represents the number of oocytes.
Within a column, values with different alphabets differ significantly by chi-square ($P < 0.05$).
[c,d] differ between the method of embryo production (IVF vs. PA) at the same origin of oocytes and maturation medium.
[x,y] differ between the maturation medium (EGF vs. MIX) at the same method of embryo production and origin of the oocyte.
[e,f] differ between the origin of oocytes (LOPU vs. slaughterhouse) at the same method of embryo production and maturation medium.
The percentages of cleavage, blastocysts (Bl), and hatched embryos in relation to the total blastocysts are indicated (mean ± SEM).
Abbreviations: COC, cumulus oocyte complex; LOPU, laparoscopic ovum pick up; PA, parthenogenetic activation; SEM, standard error of the mean.
[a] EGF: 10 ng/mL epidermal growth factor and 100 μM cysteamine.
[b] MIX: 10 ng/mL EGF, 5 UI/mL hCG, 10 UI/mL eCG, 19 ng/mL IGF-1, 2.2 ng/mL FGF, 5 μg/mL insulin, 5 μg/mL transferine, 5 ng/mL selenium, 90 μg/mL L-cysteine, 0.1 mM β-mercaptoethanol, 75 μg/mL vitamin C, 720 μg/mL glycine, 0.1 mg/mL glutamine, and 110 μg/mL pyruvate.

present study had the expected cell number. These data indicate that it is possible to produce high quality goat embryos with similar results when using complex, semi-defined, or simplified maturation media for slaughterhouse oocytes. In general, the basic IVM medium is supplemented with hormones and serum [3,4]. However, all complex supplements such as serum lead to a lack of reproducibility, and should be strongly avoided, and for further comparisons in the present study, only the simplified and semi-defined media were used.

Regarding kinetics comparison between both oocyte origins, under our system, slaughterhouse immature oocytes in the beginning of IVM were already found mostly in GVBD (73%), more than LOPU oocytes (~54%, not significant (NS), suggesting that the latter would need longer time to be matured. Confirming this hypothesis, the highest metaphase II rate was already reached at 18 hours of IVM for slaughterhouse oocytes, submitted to either maturation medium, whereas LOPU oocytes matured in EGF still progressed between 18 and 22 hours. Moreover,

Table 6
Re-expansion and hatching rates of vitrified and warmed blastocysts derived from LOPU or slaughterhouse ovaries in goats submitted to different maturation media and fertilized (IVF) or submitted to PA (mean ± SEM).

Oocyte origin	IVM system	Method	Blastocyst development stage (n)	Postwarming blastocyst survival (%)				Hatched blastocysts[a] (%)
				6 h	24 h	48 h	72 h	
LOPU	EGF[b]	IVF	Expanded (11)	27 ± 12.7^{d,x,f,h}	64 ± 16.7^{d,x,f,h}	82 ± 9.6^{d,x,f,h}	73 ± 9.6^{d,x,f,h}	73 ± 9.6^{d,x,f}
			Hatched (20)	40 ± 6.9^{d,x,f,h}	50 ± 12.0^{d,x,f,h}	65 ± 20.0^{d,x,f,h}	65 ± 20.0^{d,x,f,h}	—
		PA	Expanded (18)	50 ± 11.7^{d,x,f,h}	50 ± 11.7^{d,x,f,h}	67 ± 14.3^{d,x,f,h}	61 ± 8.8^{d,x,f,h}	61 ± 8.8^{d,x,f}
			Hatched (33)	33 ± 5.8^{d,x,f,h}	55 ± 6.1^{d,x,f,h}	85 ± 3.9^{d,x,f,h}	70 ± 6.4^{d,x,f,h}	—
	MIX[c]	IVF	Expanded (13)	46 ± 8.4^{d,x,f,h}	69 ± 10.3^{d,x,f,h}	69 ± 10.3^{d,x,f,h}	77 ± 12.9^{d,x,f,h}	69 ± 10.3^{d,x,f}
			Hatched (20)	50 ± 11.0^{d,x,f,h}	70 ± 14.4^{d,x,f,h}	80 ± 9.6^{d,x,f,h}	85 ± 8.0^{d,x,f,h}	—
		PA	Expanded (20)	55 ± 21.3^{d,x,f,h}	70 ± 23.7^{d,x,f,h}	70 ± 23.7^{d,x,f,h}	70 ± 23.7^{d,x,f,h}	55 ± 21.3^{d,x,f}
			Hatched (54)	30 ± 8.2^{d,x,f,i}	56 ± 3.5^{d,x,f,h}	78 ± 7.4^{d,x,f,h}	70 ± 6.7^{d,x,f,h}	—
Slaughterhouse	EGF[b]	IVF	Expanded (15)	40 ± 3.9^{d,x,f,h}	80 ± 7.9^{d,x,f,h}	73 ± 10.5^{d,x,f,h}	73 ± 17.8^{d,x,f,h}	53 ± 13.1^{d,x,f}
			Hatched (52)	25 ± 5.0^{d,x,f,h}	65 ± 15.1^{d,x,f,h}	73 ± 13.7^{d,x,f,h}	62 ± 7.2^{d,x,f,h}	—
		PA	Expanded (25)	40 ± 10.1^{d,x,f,h}	48 ± 20.4^{e,x,f,h}	52 ± 19.7^{d,x,f,h}	52 ± 19.7^{d,x,f,h}	48 ± 20.4^{d,x,f}
			Hatched (56)	27 ± 10.2^{d,x,f,h}	46 ± 7.0^{d,x,f,h}	57 ± 7.1^{d,x,g,h}	57 ± 12.0^{d,x,f,h}	—
	MIX[c]	IVF	Expanded (20)	35 ± 11.9^{d,x,f,h}	75 ± 19.0^{d,x,f,h}	75 ± 19.0^{d,x,f,h}	70 ± 18.9^{d,x,f,h}	50 ± 22.6^{d,x,f}
			Hatched (47)	36 ± 6.5^{d,x,f,h}	47 ± 13.4^{d,x,f,i}	57 ± 13.0^{d,x,f,h}	62 ± 12.7^{d,x,f,h}	—
		PA	Expanded (29)	38 ± 14.5^{d,x,f,h}	66 ± 18.9^{d,x,f,h}	72 ± 21.0^{d,x,f,h}	59 ± 18.5^{d,x,f,h}	48 ± 14.0^{d,x,f}
			Hatched (60)	23 ± 4.7^{d,x,f,h}	50 ± 8.8^{d,x,f,h}	68 ± 6.8^{d,x,f,h}	73 ± 3.5^{d,x,f,h}	—

Within a column, values with different alphabets differ significantly by chi-square ($P < 0.05$).
[d,e] differ between the method of embryo production (IVF vs. PA) at the same blastocyst stage, origin of oocytes and maturation medium.
[x,y] differ between the maturation medium (EGF vs. MIX) at the same blastocyst stage, method of embryo production and origin of the oocyte.
[f,g] differ between the origin of oocytes (LOPU vs. slaughterhouse) at the same blastocyst stage, method of embryo production and maturation medium.
[h,i] differ between the blastocyst stage (expanded vs. hatched) at the same origin of oocytes, maturation medium and method of embryo production.
n represents the number of blastocysts evaluated.
Abbreviations: LOPU, laparoscopic ovum pick up; PA, parthenogenetic activation; SEM, standard error of the mean.
[a] The hatching rate was calculated based on the number of vitrified blastocysts, just in the groups that were expanded before vitrification.
[b] EGF: 10 ng/mL epidermal growth factor and 100 μM cysteamine.
[c] MIX: 10 ng/mL EGF, 5 UI/mL hCG, 10 UI/mL eCG, 19 ng/mL IGF-1, 2.2 ng/mL FGF, 5 μg/mL insulin, 5 μg/mL transferine, 5 ng/mL selenium, 90 μg/mL L-cysteine, 0.1 mM β-mercaptoethanol, 75 μg/mL vitamin C, 720 μg/mL glycine, 0.1 mg/mL glutamine, and 110 μg/mL pyruvate.

slaughterhouse oocytes cultured in EGF were more advanced in meiosis than LOPU oocytes (at both 18 and 22 hours). These data generate a conflict to the theory that slaughterhouse oocytes need longer time of IVM than LOPU [10], being actually the opposite depending on the medium used. We observed a decrease (NS) in the percentage of matured oocytes at 26 hours in some groups, whereas an increase in degenerated oocytes was not observed. This difference could be because of the difference in media or culture system, as earlier suggested [8]. To make it simple, we suggest that 22 hours is an optimal time of IVM for both oocyte origins, although LOPU oocytes cultured in MIX for 26 hours had an increase of approximately 10% in maturation rate (NS). Despite 15% to 20% greater maturation rate for the slaughterhouse oocytes groups, LOPU treatments were able to achieve reasonable rates when matured for 22 hours in MIX (66%) or EGF (72%), greater than 56% [25] and 49% [26] but still lower than 85% [27]. Similar maturation rates were obtained between good and bad quality oocytes recovered by LOPU and in some groups for slaughterhouse oocytes, suggesting that regarding *in vitro* conditions grade 3 oocytes may be used to enhance the number of matured oocytes for further IVF.

To check the developmental competence of both sources of oocytes, we conducted further experiments comparing IVF and PA. Our group recently demonstrated that it was possible to enhance the number of blastocysts by some modifications on IVF system, reaching up to 54% of initial oocytes developing to the blastocyst stage. However, this experiment was performed using only slaughterhouse oocytes [9]. The same IVF conditions were used in the present study. Under our experimental conditions, although both maturation media were suitable for IVM, and IVF conditions were appropriate for slaughterhouse oocytes, LOPU-derived oocytes showed very low cleavage rate after IVF (~39%), significantly different from slaughterhouse oocytes (~67%). The high cleavage rates obtained by PA after IVM of LOPU oocytes in both maturation media (83%–93%) indicate that oocytes from LOPU have similar competence for development than slaughterhouse ones. This reflects a lower receptivity of LOPU oocytes to fertilization, related to the intrinsic quality of these oocytes, to postmortem changes in oocyte responsiveness in slaughterhouse group [6] or to inappropriate IVM conditions. Laparoscopic ovum pick up oocytes may have specific requirements during IVM or IVF to be able to reach high fertilization rate, even when nuclear maturation rate is high. During embryo development to the blastocyst stage, parthenogenetic embryos are comparable with IVF embryos [28], or even more competent than IVF ones, as reported in goats [29]. Corroborating to this argument, the cleavage rate was significantly greater in all four groups of PA embryos, compared with IVF ones. It is noteworthy that if we already had some signs that both oocyte origins have different requirements during IVM [6], the present study is the first report, which proposes that the conditions in IVF should also be adapted for LOPU oocytes.

The ability of the blastocyst to quickly restore its functionality in terms of blastocelic cavity re-expansion after vitrification and warming can be considered as a reliable marker for embryo quality and developmental potential [27]. Therefore, we evaluated the quality of the embryos produced in the different systems by looking at their resistance to cryopreservation. The survival rates of vitrified-thawed embryos were neither influenced by oocyte origin, maturation medium, nor method of embryo production. The survival rates were similar to the 63% previously reported in goat embryos [27]. It has been clearly demonstrated in cattle that although the rate of success of IVP in terms of blastocysts yield rely on oocyte intrinsic quality and maturation conditions, the quality of the resulting blastocysts (cryosurvival, viability) relies on the conditions encountered during early development [30]. Thus, it is possible to assume that our embryo culture method was adapted to support the development of the oocytes that have been able to mature and be fertilized properly, independent of their origin.

4.1. Conclusions

In conclusion, under our experimental conditions, similar maturation, fertilization and embryo development rates were obtained when using complex, semi-defined, or simplified maturation media in slaughterhouse oocytes. Slaughterhouse and LOPU-derived oocytes may have different IVM kinetics, with the latter requiring more time, depending on the IVM medium. Laparoscopic ovum pick up and slaughterhouse oocytes have similar intrinsic quality, reaching similar developmental competence in our control system using PA, whereas LOPU oocytes may be less competent for fertilization in our IVM–IVF conditions. Finally, the embryos produced by IVF or PA have similar survival rates after vitrification and thawing, demonstrating that although the IVM and IVF systems still need improvements to enhance embryo yield, the IVD step is able to generate good quality embryos from LOPU-derived oocytes. The present study has demonstrated a substantive advance in goat IVP, but further research are required for identifying specific conditions allowing efficient IVM and IVF of goat LOPU-derived oocytes.

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