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Research Article

Morphological and Functional Characterization of Cow Oocytes for Assisted Reproduction Techniques

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Abstract

In *in vitro* production of embryos (IVP), oocytes from the ovaries of slaughterhouse animals are commonly used. However, the quality of these oocytes is highly variable due to unknown category and condition of the slaughtered females. As a result, a heterogeneous population of oocytes with varying capacities for maturation and supporting embryonic development is obtained. The aim of this study was to investigate the morphological and biological characteristics of ovaries and oocytes from slaughtered bovines in order to establish selection criteria for oocytes to be used in IVP. To achieve this, the ovaries were categorized based on the presence or absence of structures such as Corpus Luteum (CL), Dominant Follicle (DF), both (CL+DF), or none (NO). The number and diameter of the follicles were also determined and grouped into three categories: <3 mm, 3-8 mm, or >8 mm. The Cumulus-Oocyte Complexes (COCs) obtained through follicular aspiration were analyzed for several parameters. These included, oocyte coverage by the Cumulus Cells (CC), the number of layers of CC, compaction and thickness of the CC layers. In denuded oocytes, the area, perimeter and diameter of the ooplasm as well as the thickness of the Zona Pellucida (ZP) were measured. Additionally, staining with Hoechst 33342, propidium iodide, or Rhodamine 123 was performed to analyze nuclear and metaphase morphology, cell viability, and mitochondrial functionality, respectively. All these determinations were made on photomicrographs captured using a camera attached to an inverted microscope and processed using the Fiji software. Statistical analysis was conducted using ANOVA and Bonferroni Test. The results revealed a predominance of ovaries with CL+DF, and in terms of follicular size, the <3 mm category exhibited the highest frequency of follicles, followed by the 3-8 mm category. Approximately 87.83±6.51% of the COCs showed complete coverage by CC; with 69.66±7.86% of the CC exhibiting compaction and 21.92±7.86% being slightly relaxed. A correlation was observed between the variable of CC layers and CC diameter (quantitative), with 91.42% of COCs having maximum diameters. Approximately, 83.11±4.27% of the oocytes displayed homogenous ooplasm, with the following measurements: area 12±0.37µm^2, perimeter 387.5±10µm, diameter 123±1.1µm, and ZP thickness 12.2±0.17µm. Moreover, 97.61± 0.11% of the oocytes were found to be viable, with no alterations observed in nuclear morphology and 100% displayed mitochondrial and metaphase activity. In conclusion, the ovaries and their corresponding COCs obtained from slaughtered females exhibit characteristics indicative of good quality for use in *in vitro* procedures.

Introduction

In vitro embryo production (IVP) has been utilized for both productive and scientific purposes, including the generation of Somatic Cell Nuclear Transfer (SCNT) cloned animals, transgenic animals, and embryonic stem cells [1]. The efficiency of IVP in livestock species, as measured by the proportion of immature oocytes reaching the blastocyst stage, rarely exceeds 30-40%. This implies that a significant percentage of oocytes do not develop after maturation [2]. In bovines, one of the reasons for the low development rates of *in vitro* matured oocytes is their degeneration. Since cows are mono-ovulatory, most oocytes used for IVP are prone to degeneration [3]. The quality of oocytes is highly variable, especially considering that the ovaries used in most cases come from slaughterhouses, and the characteristics of their origin (such as the identity or genetic merit of the donor, stage of the estrous cycle, and follicular wave stage) are often unknown [4]. Consequently, it is common to obtain a heterogeneous population of oocytes that possess varying capacities for maturation and early embryonic development after fertilization. This variability is commonly referred to as developmental competence or oocyte quality [2]. To enhance the efficiency of IVP, it is crucial to evaluate the maturation capacity and functional state of oocytes before *In Vitro* Maturation (IVM) [5].

Current evaluation criteria for the selection of oocytes or embryos are based on the number of blastomeres, proportion of apoptotic cells, and genetic and epigenetic studies. However, these techniques are invasive and have endpoint limitations, hindering the continuity of the embryonic development process. More sophisticated procedures, such as qRT-PCR, global transcriptomic analysis, and proteomic analysis, have been explored. Nevertheless, the lack of a rapid method that produces reliable results presents challenges in implementing these technologies [2]. For productive purposes, it is necessary to employ non-invasive methods to assess oocyte competence, such as morphological evaluation [6]. It is widely accepted that oocytes with homogeneous brown or brownish ooplasm, surrounded by a compact shell and multiple layers of cumulus cells, are suitable for IVM [7]. Although morphological evaluation is a useful method, it is inherently subjective, and the results can vary depending on the experience of each technician. Therefore, it is important to incorporate additional quantitative criteria to minimize discrepancies between laboratories [6]. Some determinations rely on measurements of physical parameters that can be easily and quickly assessed using microscopy, such as ooplasm diameter or area [8,9] and ZP diameter [6,10].

Therefore, by characterizing bovine ovaries and oocytes obtained from slaughterhouse animals, we can improve the identification and selection of oocytes with higher chances of development, ultimately leading to an increase in IVP efficiency. Hence, the objective of this study is to characterize bovine ovaries and oocytes based on non-invasive morphological and biological parameters to facilitate future tests for *in vitro* embryo production.

Materials and Methods

Experimental design

The experimental design is summarized in Figure 1. Initially, the morphological characterization of ovaries obtained from a slaughterhouse was conducted, categorizing them based on the presence of different ovarian structures such as corpus luteum, dominant follicle, both structures, or neither. Additionally, the number of follicles per ovary was counted and categorized into three diameter ranges (0-3 mm, 3-8 mm, and >8 mm). The results were obtained from five independent repetitions. For subsequent characterizations, the follicular aspiration technique was utilized to obtain oocytes. The oocytes were then selected using a stereomicroscope and observed and photographed using an inverted microscope coupled with a camera. The Cumulus-Oocyte Complexes (COCs) were morphologically evaluated based on the presence of cumulus cells, the qualitative thickness of the cumulus cell layers surrounding the oocyte, the compactness of the layers, and the homogeneity of the ooplasm. The results were obtained from four independent repetitions.

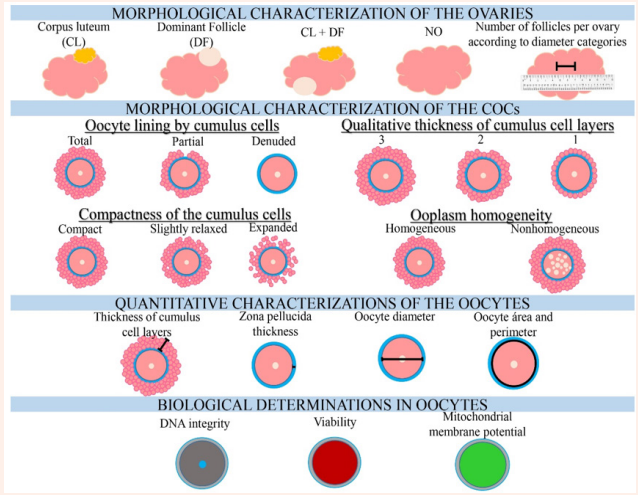

Figure N° 1: Experimental design. Image designed in the Biorender software (www.biorender.com).

For quantitative characterization, the aspirated COCs were treated with hyaluronidase to remove the cumulus cells. From the microphotographs obtained, the following measurements were taken using Fiji software [11]: quantitative thickness of the cumulus cell layers surrounding the oocyte, area, perimeter, and diameter of the oocyte, and thickness of the zona pellucida. Finally, independent staining with Hoechst, Rhodamine 123, and Propidium Iodide were performed on denuded oocytes to assess DNA integrity, mitochondrial membrane potential, and viability, respectively.

Ovary collection: Bovine ovaries were collected from the Coronel Moldes Slaughterhouse (Río Cuarto plant, Río Cuarto, Córdoba, República Argentina), which is situated approximately 10 km from the laboratory. The ovaries were transported in a thermal container at a temperature of around 25 °C. Upon arrival, the ovaries were washed with a 0.9% NaCl solution.

Morphological classification of ovaries: The ovaries were morphologically classified based on the presence or absence of ovarian structures as follow: CL (Corpus Luteum): ovaries with a corpus luteum, DF (Dominant Follicle): ovaries with a dominant follicle, CL-DF: ovaries with that had both structures, and NO: Ovaries where neither CL nor DF were present. For each individual ovary, the number of follicles falling into three separate diameter categories was counted: 0 to 3 mm, 3 to 8 mm and more than 8 mm.

Collection of cumulus-oocyte complexes (COCs): COCs were obtained using the syringe aspiration method. For this, an 18G needle coupled to a 10 ml syringe was used. The follicles were punctured by directing the needle from inside the ovarian parenchyma and aspirating multiple follicles without removing the needle. Follicles of various sizes visible on the ovarian surface were punctured as per the requirements of the experiment. The follicular fluid obtained was collected in 50 ml conical tubes, which were protected from light. The collected sample was allowed to settle for 10 minutes, after which the bottom portion was aspirated to collect the COCs. The remaining follicular fluid was centrifuged and the supernatant used for the identification of COCs under a stereomicroscope [12].

Morphological characterization of COCs: The aspirated COCs were subjected to two washes in the centrifuged follicular fluid. Subsequently, they were visualized and photographed using an Eclipse E-300 inverted microscope (Nikon, Tokyo, Japan) with an attached Nikon camera. The captured microphotographs of each registered COC were qualitatively visually characterized by the same technician based on the following criteria:

a. Oocyte coating by Cumulus Cells (CC): Oocytes that were completely surrounded by CC were classified as "totally covered." Those with discontinuity in CC layers were categorized as "partially covered," and oocytes without CC were labeled as "naked."

b. Qualitative thickness of the CC layers: COCs with the most layers of CC were given a score of "3". Those with an intermediate number of layers received a score of "2," and COCs with only 1 or 2 CC layers were scored as "1."

c. CC compaction: CCs that exhibited a close association with each other were classified as "compact." Those that showed slight relaxation, with some areas of more relaxed CC, were labeled as "slightly relaxed." CCs that appeared loosely associated and held together by a "sticky" matrix were categorized as "relaxed."

d. Ooplasm homogeneity: Oocytes with granulations homogeneously distributed in their cytoplasm were classified as "homogeneous." Oocytes with agglomerated granulations forming black dots, vacuoles, or retracted cytoplasm were labeled as "nonhomogeneous."

Microscopy images were also analyzed using Fiji software. The following measurements were performed:

A. Thickness of the CC layers: The thickness of the CC layers surrounding each COC was measured from the refringence of the Zona Pellucida (ZP) of the oocyte to the last CC layer that completely surrounded it.

B. Area, perimeter, and diameter of the ooplasm: After treatment with hyaluronidase enzyme (Sigma, H3884-50MG, 0.5 mg/ml) for 5 minutes, microphotographs of the oocytes were taken. Subsequently, the parameters of diameter, area, and perimeter were measured (excluding the ZP).

C. ZP thickness: Similar to the previous determination, the thickness of the ZP was measured in naked oocytes.

Biological characterization of COCs

Several biological parameters were analyzed in the collected oocytes:

a. DNA integrity by Hoechst 33342 stain: After the hyaluronidase treatment, the oocytes were immersed in a solution of Hoechst 33342 (Sigma-Aldrich) at a concentration of 1 µg/ml for 10 minutes in the dark. Subsequently, they were observed under an epifluorescence microscope using a filter range of excitation 360±40 nm and fluorescence emission 460±50 nm.

b. Viability assay by vital staining with propidium iodide (PI): PI (5 µg/ml, Sigma-Aldrich, St. Louis, Missouri, USA) is a dye that binds to double-stranded DNA but is excluded from cells with intact plasma membrane. The staining process was the same as that performed with Hoechst 33342. Images were acquired using fluorescence microscope with an excitation wavelength of 568 nm and an emission range of 580-660 nm.

c. Mitochondrial membrane potential (ΔΨm) by Rhodamine 123 (R123) staining: ΔΨm serves as an indicator of mitochondrial activity since the membrane plays a vital role in ATP production, redox balance, cell signaling, and metabolism. Oocytes were incubated with 10 µg/ml R123 in the dark for 15 minutes at 37 °C. Fluorescence microscopy was used to capture images with an excitation wavelength of 488 nm and an emission range of 515-575 nm. The Fiji software was employed to analyze the fluorescence intensity of each oocyte. Subsequently, the Corrected Total Cell Fluorescence (CTCF) was calculated for each oocyte using a previously described method (https://theolb.readthedocs.io/en/latest/imaging/measuring-cell-fluorescenceusing-imagej.html).

Statistical analysis

Each determination was conducted in triplicate, with some trials being quadruplicated or quintuplicated. The obtained data were analyzed using the InfoStat program [13], and post-hoc comparisons were performed using Bonferroni's test. The values are presented as means ± SEM (standard error of the mean). A significance level of $p < 0.05$ was considered statistically significant.

Results and Discussions

It has been widely recognized that the intrinsic quality of the oocyte is a critical limiting factor in blastocyst production rates. Controlling variables such as the stage of the estrous cycle, stage of the follicular wave, and origin of the ovaries becomes challenging when samples from slaughterhouses are used. Therefore, it becomes necessary to characterize the ovaries and oocytes, as the population is heterogeneous, leading to significant variability in oocyte quality.

Ovarian characterization

The presence of structures associated with the estrous cycle and the size of follicles in the ovaries appear to be linked to the competence of oocytes for embryonic development. Consequently, a characterization of the ovaries obtained from the Coronel Moldes slaughterhouse (Río Cuarto, Córdoba, República Argentina) was conducted based on the presence of Corpus Luteum (CL), Dominant Follicle (DF), both structures (CL+DF), or neither (NO). Additionally, the size of the follicles observed on the surface of the ovary, which were subsequently aspirated, was also considered. Figure N° 2 (A) provides a summary of the mean ± SEM indicating the presence or absence of ovarian structures related to the estrous cycle. The results revealed that ovaries containing both Corpus Luteum (CL) and Dominant Follicle (DF) structures (42.71 ± 8.82%) had the highest proportion compared to those with only one type of structure (CL: 14.16 ± 4.55%; DF: 23.90 ± 5.09%) or none (19.22 ± 3.76%). The importance of conducting this characterization lies in establishing a simple and non-invasive method to optimize the selection of competent oocytes for future *in vitro* reproductive technologies.

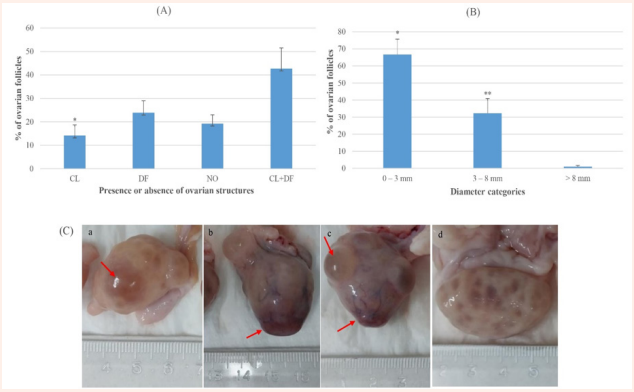

Figure N° 2: Morphological characterization of the ovaries. (A) Percentage of ovaries according to the presence of CL, DF, both CL+DF structures or their absence (NO). *p<0.05 vs percentage of ovaries with CL+DF. Values indicate means ± standard error of 5 experiments. (B) Number of follicles per ovary according to diameter categories (0-3 mm, 3-8 mm and ≥8 mm). *p<0.05 vs percentage of ovaries with follicles of size 3-8 mm and vs > 8 mm. **p<0.05 vs percentage of ovaries with follicles of size 0-3 mm and vs > 8 mm. Values indicate means ± standard error of 5 experiments. (C) Photographs of the ovary classification carried out according to morphology. The red arrows indicate the different ovarian structures a) DF; b) CL; c) CL-DF; d) NO.

Previous studies have shown varying results in the selection of ovaries based on the presence of these structures. For example, Varisanga et al. [14] reported a negative effect on the development of bovine embryos produced by *In Vitro* Fertilization (IVF) when a dominant follicle was present. Other studies [15,16] demonstrated negative effects on oocyte competence in ovaries with corpus luteum. Conversely, Manjunatha et al. [17] concluded that oocyte development was highest in pairs of ovaries with a corpus luteum and without a dominant follicle. Despite these discrepancies, selecting ovaries based on the presence of structures related to the estrous cycle may aid in accessing oocytes with improved developmental competence for *in vitro* technologies. However, further investigation is required to determine the positive or negative effects of ovarian structures on oocyte competence and subsequent embryonic development [2].

The size of ovarian follicles is anotherr criterion commonly used to obtain high-quality Cumulus-Oocyte Complexes (COCs) [2,8]. The bar graph in Figure N° 2 (B) displays the percentages found for the three categories established after performing the corresponding statistical analysis (ANOVA and Bonferroni test) using five independent repetitions. The majority of follicles found in the ovaries corresponded to the size range of 0-3 mm, accounting for a percentage of 66.71 ± 8.97%. The next highest proportion was observed in follicles with a diameter between 3-8 mm, representing 32.31 ± 8.63%, while follicles larger than 8 mm comprised only 0.96 ± 0.67% of the total. The size of the follicles determines the method for obtaining oocytes and their maturation. *In vivo*, oocytes that resume meiosis successfully typically originate from dominant follicles with a size of approximately 15 mm [18]. For the selection of potentially competent Cumulus-Oocyte Complexes (COCs) for *in vitro* maturation, small (3-5 mm) and medium (6-10 mm) antral follicles are commonly utilized [8]. In the Hansen Laboratory Protocol [19], ovaries from slaughterhouses are classified as "good" when they contain a large number of small and medium-sized follicles, which are then used to obtain COCs for *in vitro* maturation. Pavlok et al. [18] categorized COCs into three ranges of follicle size: small (less than 2 mm), medium (2-4 mm), and large (4-8 mm in diameter). Their study revealed that oocytes from medium and large follicles exhibited greater developmental potential compared to those from small follicles. This suggests that the follicles visible in the cortex of ovaries obtained from animals at the local slaughterhouse can be predominantly aspirated and used for *in vitro* maturation, *In Vitro* Fertilization (IVF), and embryo culture, as more than 90% of them fall within appropriate size ranges.

COC characterization

To further characterize the samples, we analyzed the distribution of Cumulus Cells (CC) surrounding the oocytes and classified them into three categories: total coverage (oocytes completely surrounded by cumulus cells in their entire perimeter), Partial coverage (oocytes with cumulus cells present, but with some areas showing discontinuity in the cumulus cell layers), naked oocytes (oocytes that did not have cumulus cells surrounding them). The data obtained from this analysis is presented in Figure N° 3. The analysis of cumulus cell (CC) distribution revealed that approximately 90% of evaluated oocytes were completely covered by CC (87.83 ± 8.74%), while 10.27 ± 6.88% showed partial coverage, and only 1.90 ± 1.90% were denuded. In the context of *in vitro* embryo production, good quality COCs typically exhibit certain morphological characteristics such as compact and complete CC shells, a homogeneous ooplasm with fine granulation, and a brown color [19,20]. The high percentage of COCs with total coverage observed in the raw material used in this study is a promising starting point for their utilization in future *in vitro* embryo production protocols (Figure N° 4).

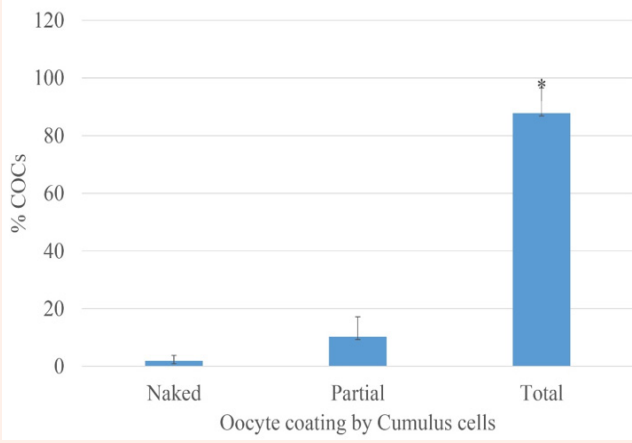

Figure N° 3: Bar graph depicting the percentages of COCs according to oocyte coverage by cumulus cells. *p<0.05 vs percentage of denuded COCs and vs percentage of COCs partially covered by cumulus cells. The values represent the means ± standard error of four experiments.

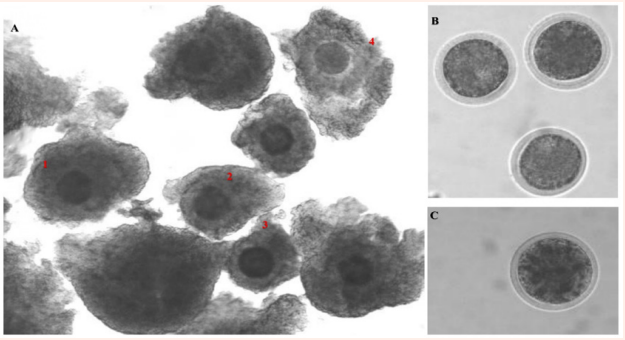

Figure N° 4: Photomicrographs of different classes of oocytes based on their morphology. A) Examples of COCs characterized as 1- completely surrounded by CC, appearing compact and with the maximum number of CC layers; 2- COC with full coverage, compact CC and with an intermediate number of CC layers; 3- COC with full coverage and the minimum number of CC layers; 4- COC with full coverage, but with slightly expanded layers of CC. B) The images show oocytes with a homogeneous ooplasm; C) oocyte with non-homogeneous ooplasm, characterized by the presence of black granules.

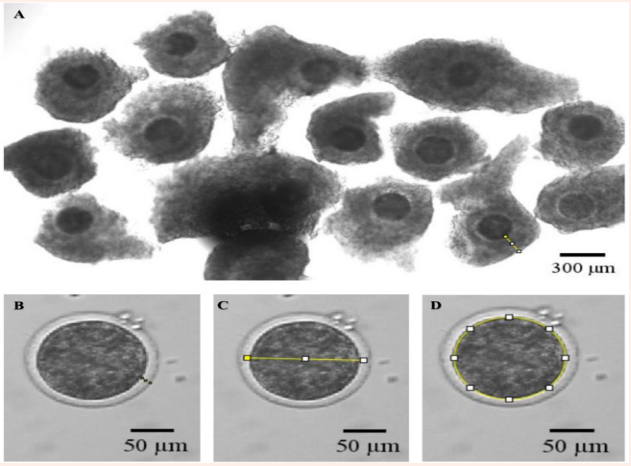

Figure N° 5: Photomicrographs illustrating the measurements obtained through the Fiji software are shown for: A) thickness of the CC cover; B) ZP thickness; C) ooplasm diameter; D) ooplasm area and perimeter.

Subsequently, we investigated whether the qualitative thickness of the CC layers was related to the quantitative thickness of the CC layers (Figure N° 5 A). As described in the Materials and Methods, COCs were categorized as "1" for those with the least amount of layers, "2" for intermediate amount, and "3" for those with considerable CC layers. We aimed to determine if there was a correlation between these two characteristics, specifically the average thickness of CC layers for COCs classified as 1, 2, and 3. The results indicated that the average thickness of CC layers for COCs classified as 1 was 0.05 mm, for 2 it was 0.08 mm, and for 3 it was 0.13 mm. Furthermore, to statistically validate if there were differences among these three averages and to group the observed characteristics (number of layers 1, 2, and 3) into three quantitative categories based on average thickness (0.05, 0.08, and 0.13 mm), variance analysis (ANOVA) was performed using the InfoStat program. Table N° 1 displays the obtained values. The results show a significant difference in the average thickness of the Cumulus Cell (CC) layers among the three groups, allowing for the establishment of three different categories based on the quantitative and qualitative variables of CC layer thickness. Specifically, qualitative variable 1 corresponds to an average thickness of CC layers of 0.05 mm, variable 2 corresponds to 0.08 mm, and variable 3 corresponds to 0.13 mm. The significance of these findings lies in the transformation of a qualitative and subjective selection characteristic of Cumulus-Oocyte Complexes (COCs) into a quantitative and objective one. This measurement can serve as a new non-invasive marker for potentially competent COCs, as criteria for selecting and classifying oocytes vary among researchers and can be subjective and dependent on the evaluator's experience [2].

Table N° 1: Mean ± SEM of the thickness determinations of the CC layers of COCs with "1", "2" or "3" layers amount.

Variable	n	Mean ± SEM
Thickness of CC layers "1"	18	0.05 ± 0.01 mm*
Thickness of CC layers "2"	70	0.08 ± 0.004 mm**
Thickness of CC layers "3"	122	0.13 ± 0.003 mm

Additionally, the results demonstrate that the ovarian samples used in the study had 58.09% of COCs belonging to the category with the highest average thickness of CC layers and 33.33% in the category with an average thickness of 0.08 mm, making up a total of 91.42%. Thus, the samples from the slaughterhouse can be concluded to be of excellent quality based on the intrinsic characteristics analyzed. Regarding the compaction degree of CCs in the aspirated COCs, three categories were distinguished: compact, slightly relaxed, and relaxed (Figure N° 4). The statistical evaluation, based on four independent repetitions, yielded the following mean values: compact 69.66 ± 7.86%, slightly relaxed 21.92 ± 7.86%, relaxed 8.41 ± 7.86%. The analyzed samples predominantly consisted of COCs with compact CCs, with a significantly higher percentage compared to the slightly relaxed and relaxed categories. According to COC selection criteria proposed by authors such as Aguila et al. [2] and Madison et al. [21], both COCs with compact CCs and slightly relaxed/expanded CCs are considered good quality for subsequent *in vitro* tests. Taking these principles into account, the ovaries from the Coronel Moldes slaughterhouse rendered 91.58% of COCs with compact or slightly relaxed CCs, which are considered of good quality for use in *in vitro* embryo production technologies. This implies that around 90% of the aspirated COCs from the studied samples possess the desired characteristics in terms of CC coverage, CC thickness, and compaction quality for selection in *in vitro* tests.

In terms of ooplasm, oocytes with uniformly granulated cytoplasm without clear spaces are typically selected for *in vitro* maturation [19]. Based on this criterion, the oocytes were analyzed for cytoplasmic homogeneity, resulting in 83.11 ± 4.27% of oocytes with homogeneously granulated cytoplasm, indicating good quality according to the analyzed characteristic (Figure N° 4). Previous studies, such as those conducted by Leibfried [7], suggest that oocytes with granules that aggregate or are unevenly distributed, leading to the merging of black bodies or an uneven filling of the zona pellucida, exhibit signs of atresia or degeneration and possess limited or no developmental competence. However, more in-depth studies, such as those conducted by Nagano et al. [3], have observed that black granules do not always indicate atresia but may be due to lipid accumulations, which are associated with a high potential for development. They further concluded that a dark ooplasm indicates lipid accumulation and good developmental potential, while a light-colored ooplasm indicates low

organelle density and a black ooplasm indicates aging, both with low developmental potential. Therefore, although high percentages of oocytes with homogeneous cytoplasm were obtained, further investigation is needed to determine if the black dots observed correspond to lipid droplets or are indicative of atresia in the remaining 17% of oocytes with non-homogeneous cytoplasm.

In addition, various physical parameters were determined for the naked oocytes to further characterize them and explore potential relationships between morphology and quality (Figure N° 5). Using the Fiji software, the following measurements were obtained: average ooplasm area of 12 ± 0.37 µm², average ooplasm perimeter of 387.5 ± 10 µm, average ooplasm diameter of 123 ± 1.1 µm (excluding the zona pellucida) (Figure N° 5, C and D), and average thickness of the Zona Pellucida (ZP) of 12.2 ± 0.17 µm (Figure N° 5 B). Regarding the ooplasm diameter parameter, which is commonly used as a selection criterion for potentially competent oocytes, Otoi et al. (1997) [9] reported an average diameter of 114.0 ± 4.8 µm. They found that oocyte diameter is related to developmental competence, with oocytes acquiring full meiotic competence at a diameter of 115 µm, but a diameter of 120 µm being required for blastocysts to achieve full developmental competence. On the other hand, Hyttel et al. [8] reported different results, where oocytes needed a diameter of approximately 100 µm to acquire full competence for meiotic resumption, and a diameter of around 110 µm to maintain embryonic development competence.

Studies conducted on human oocytes have also demonstrated a relationship between ZP thickness and developmental capacity [10]. Similar results have been observed in other species such as pigs and buffalos, suggesting that this trend may be conserved across different species, including cattle [6]. Due to these subtle variations observed in different systems, it was necessary to characterize the intrinsic values of the oocytes obtained from the Coronel Moldes slaughterhouse samples to better understand their specific characteristics. In terms of nuclear morphology and oocyte metaphase arrest, no alterations were observed in the nuclear morphology of any oocyte (Figure N° 6), and 100% of the oocytes exhibited metaphase arrest. This indicates that the oocytes obtained from the ovaries of animals from the local slaughterhouse were in a developmentally competent state at the time of collection.

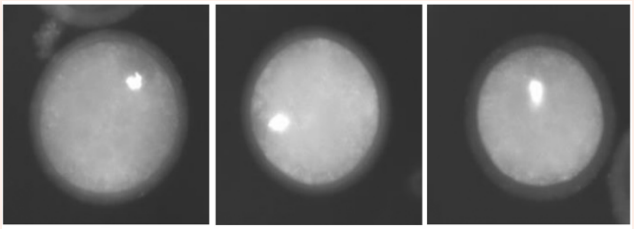

Figure N° 6: Hoechst-stained oocytes displaying no alterations in nuclear morphology.

Regarding cell viability, only 2.39 ± 0.11% of the oocytes stained with Propidium Iodide (PI) emitted fluorescence, indicating cell death, while the majority of the population, 97.61 ± 0.11%, remained viable. This demonstrates that the vast majority of the oocytes used in the study were alive and maintained intact plasma membranes. Furthermore, all the oocytes analyzed exhibited mitochondrial activity as they were stained by Rhodamine 123 (R123) and displayed fluorescence. The fluorescence intensity of R123 was measured using the Fiji software, and three subpopulations were established based on fluorescence intensity ranges: low CTCF (Corrected Total Cell Fluorescence), intermediate CTCF, and high CTCF. Interestingly, each range accounted for 33.33% of the total number of oocytes, indicating a relatively equal distribution. This suggests that a significant proportion of the oocytes (66.66%) exhibited intermediate to high mitochondrial functionality.

Conclusion

The comprehensive morphological and biological characterizations of the ovaries, Cumulus-Oocyte Complexes (COCs), and oocytes obtained from the Coronel Moldes slaughterhouse in Río Cuarto, Córdoba, República Argentina have provided valuable insights. The analysis of the ovaries revealed a predominance of samples with both Corpus Luteum (CL) and Dominant Follicle (DF) structures, indicating a higher potential for oocyte development. Furthermore, the size distribution of the follicles in the ovaries demonstrated that a significant proportion of the follicles fell within the range typically used for *In Vitro* Maturation (IVM), *In Vitro* Fertilization (IVF), and *in vitro* embryo production (IVP) experiments. This suggests a plentiful supply of Cumulus-Oocyte Complexes (COCs) for further studies. The morphological and biological determinations conducted on the COCs and oocytes indicated that approximately 90% of them exhibited good quality characteristics. These included complete coverage by cumulus cells, desirable thickness of cumulus cell layers, compactness of cumulus cells, homogeneous ooplasm, absence of nuclear alterations, arrested in metaphase I, high viability, and mitochondrial functionality. These findings validate the suitability of the ovaries from the local slaughterhouse as a reliable source of high-quality COCs for use in IVP trials.

In summary, the characterization of both the ovaries and their derived COCs and oocytes from the Coronel Moldes slaughterhouse has provided valuable insights and demonstrated their potential for use in *in vitro* reproduction technologies. The abundance of good quality COCs from these ovaries opens up opportunities for further advancements in IVM, IVF, and IVP research.

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