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Relationship between chromatin configuration and in vitro maturation ability in guinea pig oocytes

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Abstract

Background: Germinal vesicle (GV) chromatin configurations of oocytes are proposed to be related to oocyte competence and may reflect the quality of oocyte. Currently, a limited number of published studies investigated the GV chromatin configurations of guinea pig oocytes.

Objective: In this study on the in vitro maturation (IVM) of guinea pig oocytes, we examined the changes in their GV chromatin configurations during meiotic progression.

Methods: Based on the degree of chromatin compaction, the GV chromatin configurations of guinea pig oocytes could be divided into three categories depending on whether the nucleolus-like body (NLB) was surrounded or partly surrounded by compacted chromatin, namely the uncondensed (NSN), the intermediate type (SN-1) and the compacted type (SN-2).

Results: The percentage of cells displaying the SN-2 configuration increased with the growth of guinea pig oocytes, suggesting that this configuration presents the potential for maturation in oocytes. Oocytes derived from larger follicle exhibited increased meiotic potential. Serum starvation affected the GV chromatin configurations of guinea pig oocytes.

Conclusions: Collectively, these results suggest that the SN-2 type might be a more mature form of configuration in guinea pig oocyte, whose proportion was associated with the follicle size and susceptible to the environment (e.g. serum concentration).

KEYWORDS
chromatin configuration, competence of maturation, germinal vesicle, guinea pig oocyte

1 | INTRODUCTION

Embryo engineering requires a large number of high-quality mature oocytes (Moawad et al., 2013). Under natural conditions, the immature (primary) oocytes in the ovaries of mammals are typically arrested at the diplotene stage of the first meiotic division (Burks et al., 2019); at this time, the nucleus of oocyte is also called the germinal vesicle (GV). Such arrest persists until the sexual maturation, when one of the immature oocytes completes meiosis before being released as a mature oocyte during ovulation (Virant-Klun et al., 2013). With the implementation of embryo engineering technologies, an extra of mature oocytes could be obtained through the in vitro maturation (IVM) of immature oocytes or ovaries (Bogliolo et al., 2007; Cha & Chian, 1998; Hu et al., 2011; Reynaud et al., 2012; Taiyeb et al., 2020; Taiyeb et al., 2017). Studies have shown that the efficiency of IVM depends greatly on the quality of the oocyte itself, rather than the in vitro culture system (Gilchrist et al., 2016). Therefore, the efficacy in screening high-quality immature oocytes might determine the outcome of IVM. During oogenesis, oocytes regulate gene expression by altering chromatin structure, and the GV changes significantly during the growth and development of oocytes, before eventually undergoing germinal vesicle breakdown (GVBD) that enables oocytes to resume meiotic processes; these phenomena have been reported in many animals (Pan et al., 2018; Sun et al., 2004; Tan et al., 2009). During IVM of horse oocytes, changes in chromosomal configuration are characterised by a remarkable reorganisation of microtubules and microfilaments, which is essential for chromosomal alignment and segregation (Tremoleda et al., 2001). Another study aimed at exploring chromatin patterns of IVM oocytes also indicated that chromatin changes in the GV are induced during IVM (Reynaud et al., 2012). It was also demonstrated that changes of chromatin configuration in porcine oocytes can significantly affect follicle size, which has repercussions on meiosis and developmental competence of oocytes (Lee et al., 2019). Given the foregoing, GV chromatin configuration has been found to associate with the developmental ability and may serve as a criterion for determining the quality of oocytes.

There have been many studies on the GV chromatin configuration of animals such as porcine, bovine, goat and canine (Lee Et Al., 2008; Liu et al., 2006; Sui et al., 2005; Sun et al., 2004). In bovine oocytes, chromatin configuration changes and oocyte development competence are regulated by the gap junction-mediated communications (Lodde et al., 2013). Chromatin configuration and histone modification also play significant roles in the developmental competence of GV stage oocytes (Wu et al., 2015). GV chromatin configuration was reported in porcine oocytes and was found to be regulated by the MAPK activity (Sun et al., 2016). GV configuration and global DNA methylation were also reported in ovine oocytes (Cocero et al., 2019) and in various cattle breeds (Soares et al., 2020). However, systematic studies of GV chromatin configuration have not been reported in guinea pigs, an animal that shows similarities to human reproductive physiology. Studies in other animals have shown that oocytes gradually gain meiotic competence and developmental capacity as follicles grow, but oocytes isolated from follicles of the same size are still heterogeneous, which may have an impact on meiosis progression, cytoplasmic maturation and subsequent developmental capacity (Pan et al., 2018). Therefore, it is extremely important to determine the quality of oocyte by clearly describing its morphological and biological characteristics at a single-oocyte scale, before proceeding to the next step.

This study investigated the changes in chromatin configuration during meiotic progression, in order to clarify the relationship between chromatin configuration and maturation ability in guinea pig oocytes; the influence of follicle size and serum concentration on oocytes development was also investigated. The current results of chromatin conformational changes in oocytes improved our understanding on the progression of oocyte meiosis in guinea pigs at both cellular and molecular levels and set a foundation for embryo engineering involving guinea pigs.

2 | MATERIALS AND METHODS

2.1 | Collection of guinea pig oocytes

One-month-old female guinea pigs were used in this study. Every guinea pig was injected with 15 IU of human menopausal gonadotrophin for three consecutive days. On the fourth day, the guinea pigs were euthanised by intraperitoneal injection of sodium pentobarbital (150–200 mg/kg), and the ovaries were collected and washed three times with phosphate-buffered saline (PBS). The excess fat was removed with forceps and a scalpel under dissecting microscope. According to the follicle diameter (< 0.5, 0.5–0.8 or 0.8–1.2 mm), they were divided into three groups. Subsequently, the different sizes of follicles soaked in M2 medium were punctured with a syringe needle to release cumulus oocyte cell complexes (COCs), which were then collected under a stereo microscope using a custom glass capillary.

2.2 | Conformational classification of GV chromatin

The oocytes were transferred to M2 medium containing 0.1% (v/v) hyaluronidase, and the surrounding cumulus cells were removed using a custom glass capillary. The purified oocytes were transferred to M2 medium containing 0.5 μg/ml Hoechst 33342 (Sigma, St Louis, MO, USA) and incubated for 5 min at 38.5°C, in a 5% CO_2 incubator. Finally, the oocytes were placed on a glass slide and flattened with a coverslip. The morphology of the nucleolus and nuclear membrane was first observed under phase-contrast microscopy (Nikon, Tokyo, Japan), and then the GV chromatin configuration was observed under fluorescence microscopy (Nikon, Tokyo, Japan).

2.3 | Induction of oocyte IVM under different conditions

The IVM medium was supplemented with 10% (v/v) fetal calf serum (FCS) and 10 IU/ml pregnant mare serum gonadotropin (PMSG) in

TCM-199 medium (Gibco, Grand Island, NY, USA). A dozen of COCs was placed in 100 μl of medium, covered with paraffin oil and cultured at 38.5°C in a 5% CO_2 incubator. A portion of COCs were examined for IVM-mediated alterations of GV chromatin configuration after 0, 2, 4 and 6 h of culture, the rest of COCs were used for evaluation of nuclear maturation ability after 24 h of culture. In addition, to investigate the effect of serum on the GV chromatin configuration, some COCs were cultured in TCM-199 supplemented with only PMSG (FCS-free).

2.4 | Evaluation of oocytes meiotic progression

After 24 h of culture, the oocytes were removed from the COCs as described above and placed on a glass slide. The oocytes were sealed under coverslips using petrolatum and paraffin wax. The mounted slides were then immersed in a solution of ethanol:acetic acid (v/v 3:1) for at least 24 h. The oocytes were stained with Hoechst 33342 and observed under a phase-contrast microscope (Nikon, Tokyo, Japan) and classified into four categories based on GV phase/stage, namely GVBD stage, first meiosis metaphase (MI), anaphase/telophase phase (AnaI/TelI) and second meiosis (MII).

2.5 | Statistical analysis

The data were analysed using SPSS 17.0 software and expressed as mean ± standard error. The data of two groups were compared using the independent sample *t*-test; the data comparison between three or more groups was analysed by one-way analysis of variance (ANOVA), and the multiple comparisons were performed using the LSD method. If the data did not meet the conditions of one-way ANOVA, a rank sum test was used. Each experiment was repeated at least three times. $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Observations of GV chromatin configuration

After Hoechst 33342 staining, the GV chromatin configurations were observed by phase-contrast and fluorescence microscopy. According to the degree of chromatin compaction and the visibility of nucleoli and nuclear membranes, the GV chromatin configurations of guinea pig oocytes were divided into three types: the uncondensed type (NSN-type), the intermediate type (SN-1-type) and the compacted type (SN-2-type) (Figure 1). The NSN-types exhibited chromatin that did not surround the nucleolus and was instead dispersed throughout the nucleoplasmic region (Figure 1a, a'). The SN-1-type oocytes showed that a part of chromatin was condensed around the nucleolus, and some chromatin dispersed in the nucleoplasm (Figure 1b, b'). The SN-2-type oocytes showed that almost all chromatin was condensed around the nucleolus (Figure 1c, c').

3.2 | Conformational alterations of GV chromatin in oocytes during follicular growth

The changes in the GV chromatin configurations of oocytes obtained from follicles with different diameters are summarised in Table 1. The results showed that the GV chromatin configurations of oocytes derived from follicles with diameter < 0.5 mm were all NSN-type. With the growth of follicles, the proportion of NSN-type decreased ($p < 0.01$) and the proportion of SN-2-type and GVBD increased ($p < 0.01$). There was no significant difference in the percentage of SN-1-type between oocytes with follicle diameters of 0.5–0.8 mm and 0.8–1.2 mm. These results indicated that the proportion of oocytes in the SN-2 configuration increased with the growth of follicles.

3.3 | Changes in GV chromatin configuration during oocyte IVM

To further observe the changes in GV chromatin configuration during oocyte IVM, follicles with a diameter of 0.8–1.2 mm were selected and cultured for different times (0, 2, 4 and 6 h) in vitro. The results showed that during the IVM process, the proportion of NSN-type and SN-1-type of oocytes decreased, while the proportion of SN-2-type and GVBD-type increased (Table 2). This suggested that the NSN- and SN-1-types may be converted to a more mature SN-2-type during in vitro meiotic progression of oocytes.

3.4 | Association between follicle size and meiotic progression of oocytes

Table 3 lists the distribution of meiotic stages of guinea pig oocytes with different follicle sizes after 24 h of in vitro culture. The results showed that all oocytes with follicles less than 0.5 mm in diameter were in the GV phase. Although more than half (57.1%) of the oocytes with 0.5–0.8 mm diameter follicles resumed meiosis, they were arrested in the GVBD stage or MI phase. Furthermore, the oocytes with 0.8–1.2 mm diameter follicles all underwent meiosis, of which 62.0% reached the MII phase.

3.5 | Effect of serum deprivation on GV chromatin configuration

To elucidate the effect of FCS on the GV chromatin configuration during IVM, we selected oocytes with 0.8–1.2 mm diameter follicles and observed their GV chromatin configuration in response to altered serum concentrations. The proportion of NSN configurations was slightly increased in FCS (–) group after 4 h of culture, although it did not significantly alter in FCS (+) group after 2 and 4 h of culture (Table 4). As for other GV configurations, the proportions of SN-1 type and GVBD stage were significantly elevated at both the 2 and 4 h time

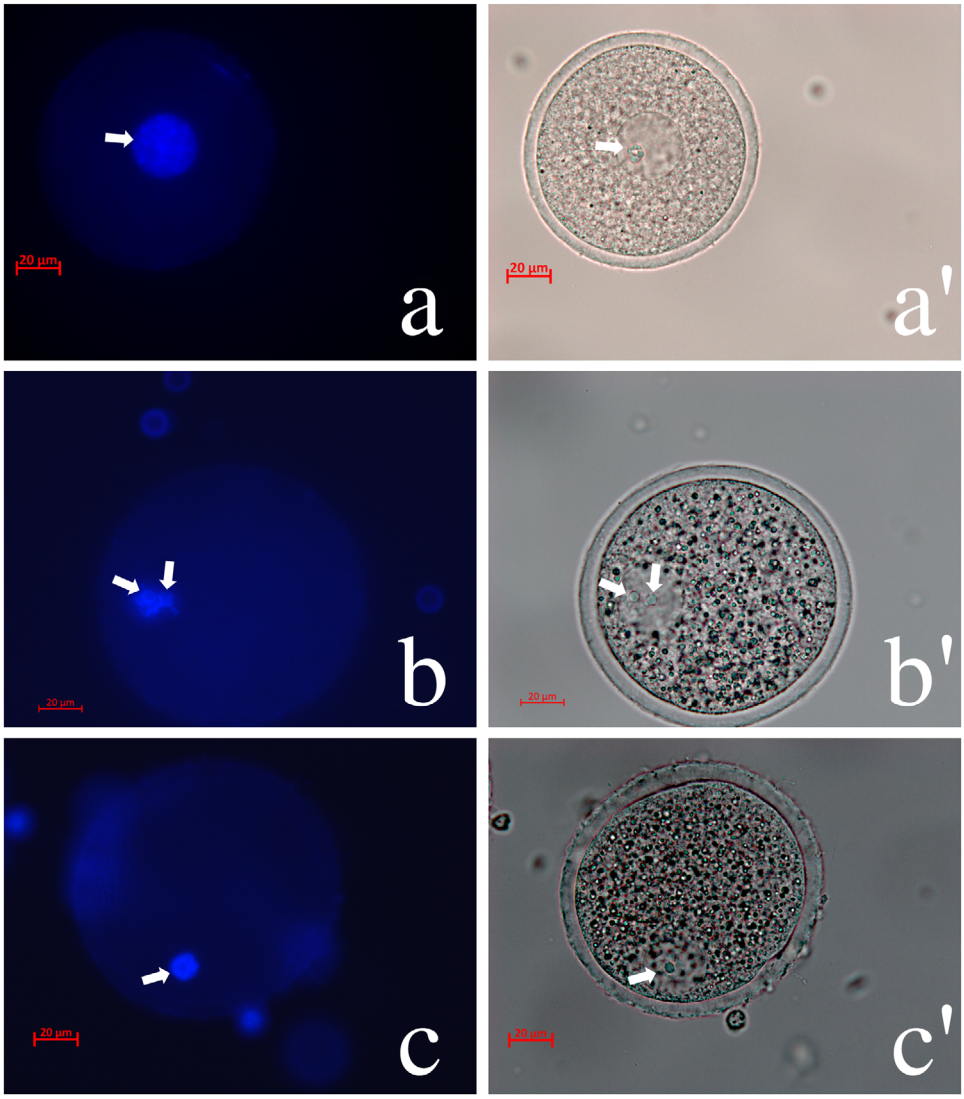

FIGURE 1 The classification of germinal vesicle (GV) chromatin configurations in guinea pig oocytes. (a, b and c) NSN/SN-1/SN-2 configuration visualised by phase-contrast microscopy, corresponding to (a', b' and c') NSN/SN-1/SN-2 visualised by fluorescence microscopy. (a and a') NSN-type (uncondensed type) exhibited chromatin that did not surround the nucleolus and that was dispersed throughout the nucleoplasmic region. (b and b') The SN-1-type (intermediate type) showed partially condensed chromatin around the nucleolus, and some chromatin dispersed in the nucleoplasm. (c and c') The SN-2-type (compacted type) showed that the chromatin was condensed around the nucleolus

TABLE 1 Configuration of germinal vesicle (GV) chromatin of guinea pig oocytes from follicles of different diameters

Follicle diameter (mm)	Oocytes observed	NSN (%)	SN-1(%)	SN-2(%)	GVBD (%)
<0.5	28	100 ± 0	0 ± 0	0 ± 0	0 ± 0
0.5–0.8	43	39.0 ± 9.6**	33.9 ± 6.2**	14.5 ± 1.4**	12.5 ± 6.3**
0.8–1.2	105	5.1 ± 3.5**##	33.1 ± 6.3**	52.4 ± 9.0**##	9.4 ± 1.4**

NSN: nonsurrounded nucleolus; SN-1: chromatin partly surrounded nucleolus; SN-2: all the chromatin surrounded nucleolus; GVBD: germinal vesicle breakdown.

**$p < 0.01$ vs. the follicle diameter < 0.5 mm group.

##$p < 0.01$ vs. the follicle diameter 0.5–0.8 mm group.

TABLE 2 Changes in configuration of GV chromatin during in vitro maturation (IVM) of oocytes collected from 0.8 to 1.2 mm follicles

Culture time (h)	Oocytes observed	NSN (%)	SN-1(%)	SN-2(%)	GVBD (%)
0	105	5.1 ± 3.5	33.1 ± 6.3	52.4 ± 9.0	9.4 ± 1.4
2	64	0 ± 0**	6.5 ± 2.6**	66.7 ± 5.6**	26.9 ± 3.2**
4	55	0 ± 0**	15.0 ± 1.3**	50.1 ± 4.7	34.9 ± 5.1**
6	72	0 ± 0**	12.8 ± 1.5**	43.1 ± 0.7	44.1 ± 1.1**

NSN: nonsurrounded nucleolus; SN-1: chromatin partly surrounded nucleolus; SN-2: all the chromatin surrounded nucleolus; GVBD: germinal vesicle breakdown.
**$p < 0.01$ vs. the control group (culture time: 0 h).

TABLE 3 Competence of nuclear maturation of guinea pig oocytes from different diameters follicles after 24 h of culture

Follicle diameter						
(mm)	Oocytes observed	GV (%)	GVBD (%)	MI (%)	AnaI/TeII (%)	MII (%)
<0.5	85	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
0.5–0.8	87	42.9 ± 4.3**	22.1 ± 2.5**	34.9 ± 6.1**	0 ± 0	0 ± 0
0.8–1.2	76	0 ± 0**##	10.7 ± 1.8**##	28.5 ± 5.47**	17.50 ± 4.4**##	42.1 ± 1.1**##

GV: germinal vesicle, GVBD: germinal vesicle breakdown stage, MI: first meiosis metaphase, AnaI/TeII: anaphase I/telophase I, MII: secondary meiosis.
**$p < 0.01$ vs. the follicle diameter < 0.5 mm group.
##$p < 0.01$ vs. the follicle diameter 0.5–0.8 mm group.

points in the absence of FCS, accompanied by reduced proportion of SN-2 type (Table 4). These results suggested that serum deprivation accelerated the progression of oocyte meiosis but prevented the conversion of oocytes from SN-1- to SN-2-type.

4 | DISCUSSION

In numerous mammalian species, chromatin configuration of GV plays a tremendous role in the oocyte competence. Until now, the chromatin configuration of GV and its potential effects on meiotic competence in guinea pig have not been reported elsewhere despite the value of guinea pig cloning for studying human diseases. In this study, we used Hoechst 33342 to stain the DNA of guinea pig oocytes. According to the degree of chromatin compaction, the GV chromatin of guinea pig oocytes was divided into three types; namely NSN, SN-1 and SN-2 types. With the growth of follicles, it is commonly observed, among most animals (except goats), that diffused chromatin gradually aggregates around the nucleolus (Hinrichs et al., 1993; Hui-Li et al., 2009). In this study, nucleoli and chromatin were clearly observed after staining with Hoechst 33342, and with the procession from NSN to SN-1, and finally SN-2 type, the extent of chromatin condensation was elevated and a clear perinucleolar ring could be observed around nucleolus-like body. Therefore, SN-2 type could be considered as a further step toward maturation in guinea pig, which was consistent with results from other animals (Pan et al., 2018; Zuccotti et al., 1998).

Here, we found that the GV chromatin of guinea pig oocytes could be classified into three types (NSN, SN-1 and SN-2 types). In ferret, three dissimilar chromatin configurations, namely fibrillar chromatin (FC), intermediate condensed chromatin (ICC) and condensed chromatin (CC) were also identified (Sun et al., 2009). In canine species, five types of chromatin configuration (types A-E) have been previously identified and the proportions of GVs of C, D and E types and those of MI and MII meiosis are applicable to the evaluation of meiotic resumption following IVM (Reynaud et al., 2012).

TABLE 4 Effects of fetal calf serum (FCS) on configuration of GV chromatin during in vitro maturation of oocytes from 0.8 to 1.2 mm follicles

Culture time (h)	FCS	Oocytes observed	NSN (%)	SN-1 (%)	SN-2 (%)	GVBD (%)
0		105	5.1 ± 3.5	33.1 ± 6.3	52.4 ± 9.0	9.4 ± 1.4
2	+	47	0 ± 0	8.6 ± 2.0	61.5 ± 3.0	30.0 ± 1.1
2	–	65	0 ± 0	22.9 ± 5.2**	25.1 ± 4.2**	52.0 ± 3.8**
4	+	55	0 ± 0	15.0 ± 1.3	50.1 ± 4.7	34.9 ± 5.1
4	–	65	1.0 ± 0.8	25.3 ± 0.8**	18.6 ± 5.4**	56.2 ± 11.8**

NSN: nonsurrounded nucleolus; SN-1: chromatin partly surrounded nucleolus; SN-2: all the chromatin surrounded nucleolus; GVBD: germinal vesicle breakdown.
**$p < 0.01$ vs. corresponding FCS (+) group.

It has been shown that in mice (Zuccotti et al., 1998; Zuccotti et al., 2010), horses (Hinrichs, 2010) and humans (Miyara et al., 2003), chromatin loops condense around the nucleolus as the follicles grow, before the fully grown oocytes can be ovulated. We studied the chromatin configuration of guinea pigs and found that with the growth and maturation of oocytes, the proportion of SN-2 configuration increased, while the proportion of NSN-type decreased gradually. These results indicated that the chromatin configuration of guinea pig oocytes was related to the maturation ability of oocytes, corroborating that the chromatin circular compaction configuration around the nucleolus, namely the SN-2-type, was more mature. Previous studies have shown that SN-type GV oocytes are more appropriate for IVF compared to NSN-type GV oocytes and that bigger follicles are correlatively filled with SN GV oocytes and exhibit higher developmental competence during IVM due to accelerated meiosis and blastocyst development (Lee et al., 2019). The mechanisms driving the NSN/SN transition chromatin configuration in oocyte development and maturation are ill-defined. In mice, it was reported that meiosis and growth competence discrepancy among the SN and NSN oocytes is due to nuclear and cytoplasmic factors and that the content of SN-type GVs are determinant for complete oocyte growth (Inoue et al., 2008). Another study indicated that MAPK activity play an important role in chromatin configuration during oocyte development (Wu et al., 2015). Thus, we stipulate that these factors are potentially involved in the mechanism of chromatin configuration in guinea pigs, but genuinely designed experiments are needed to uncover the factors driving the chromatin configuration of guinea pig oocytes in IVM.

Embryo biotechnology increasingly requires IVM oocytes. Although intensive research has been conducted, the viability of viable embryos produced in vitro is still far less than that produced in vivo. Currently, a common problem with embryos produced in vitro is that the oocyte cytoplasm is not fully formed (Gruhn et al., 2018). Studies have shown that as follicles grow, oocytes gradually gain meiotic and embryonic developmental abilities; however, oocytes obtained from the same size follicles are heterogeneous, which may explain the difference in the chromatin configurations for oocyte meiosis, cytoplasmic maturation ability and embryo development ability (Marchal et al., 2002). Therefore, it is important to measure the quality of oocytes according to the different oocyte chromatin configurations before culture. Previous reports showed that in vivo, the size of isolated follicles was correlated with oocyte quality in bovine and porcine models (Xiao et al., 2015). In the present study, we evaluated the association between follicle size and meiotic stages and found that guinea pig oocytes derived from larger follicle exhibited increased meiotic potential, especially for that in follicles with a diameter larger than 0.8 mm. Combine with a recent research on sheep follicles proposing that cumulus oocyte complexes derived from follicles with smaller size were less competent to form blastocyst (Rouhollahi Varnosfaderani et al., 2020), follicle with larger size might represent better oocyte quality. Similarly, the porcine oocytes derived from bigger follicles was correlated with SN status (Lee et al., 2019).

A previous study on reconstruction of bovine oocytes showed that serum deprivation improved cleavage rate and promoted blastocyst formation (Cho et al., 2002), suggesting that the absence of serum might be beneficial for preimplantation development of bovine oocytes. The effect of serum deprivation on guinea pig oocytes was also investigated in the present study; the results showed that the proportions of SN-1 type and GVBD stage were significantly elevated in the absence of FCS, accompanied by reduced proportion of SN-2 type. These observations suggest that the serum starvation might promote the maturation of guinea pig oocytes (elevated GVBD), and at the same time prevent the conversion of oocytes from SN-1- to SN-2-type (which might be considered as an obstacle to the meiotic process), the latter was not totally consistent with its oocyte development-promoting property as depicted in a preceding study (Cho et al., 2002); such discrepancy might be ascribed to the fact that the bovine oocytes used by Cho et al. were modified by reconstruction with cumulus or ear fibroblasts, or inter-species heterogeneity.

In conclusion, the current study reveals the relationship between the chromatin configurations and maturation ability of guinea pig oocytes in an in vitro setting and their association is susceptible to the environment (for example, serum supplementation). These observations provide valuable evidence of high-quality oocytes selection, and thus may benefit the embryo engineering involving guinea pigs.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

MY, WC and LL performed the animal experiment, interpreted the data, wrote and revised the manuscript. HZ, WG, FM, JZ and LW contributed to the drafting of the manuscript and data analysis. YS, YL and HS designed the study and revised the manuscript. All the authors have read and approved the final version of the submitted manuscript.

ETHICS APPROVAL

All animal handling procedures were approved by the local Institutional Animal Care and Use Committee of Shandong First Medical University. All animal experiments have been carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals. All animal studies were compiled with the ARRIVE guidelines.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

PEER REVIEW

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