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Association between chromatin configuration and developmental competence of germinal vesicle-stage oocytes in guinea pigs

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ABSTRACT: Large quantities of oocytes for embryo engineering are obtained through *in vitro* maturation (IVM). Although IVM oocytes are arrested at the diplotene stage of the first meiosis, they exhibit distinct quality heterogeneity, and reliable selection criteria are urgently required to improve IVM efficiency. As guinea pigs share high similarities with human reproductive physiology, they serve as a suitable animal model to investigate oocyte chromatin configuration. In this study, germinal vesicle (GV) chromatin of guinea pig oocytes was classified into five subtypes (NSN, pNSN, SN-1, cSN-1, and SN-2) based on chromatin compaction. We further explored the relationship between chromatin configurations and developmental competence by monitoring changes associated with follicular and oocyte growth, tracking chromatin dynamics during hormone-induced *in vivo* maturation, and detecting RNA transcriptional activity.Chromatin gradually transitions from NSN to SN-2 as follicles and oocytes develop. The proportion of SN-2 oocytes increases progressively during *in vivo* maturation, accompanied by declining transcriptional activity. In conclusion, SN-2 represents the most mature GV chromatin stage in guinea pig oocytes, and RNA transcriptional activity is negatively correlated with GV-oocyte maturity.

KEYWORDS: germinal vesicle stage, chromatin configuration, developmental competence, RNA transcription, guinea pig oocytes

INTRODUCTION

With advancements in embryo engineering technologies, significant milestones have been achieved in mammalian cloning, gene knockout, and transgenic animal production. However, their success rates remain suboptimal, underscoring the critical importance of research on oocyte maturation. In several mammalian species, dynamic chromatin configuration changes within the germinal vesicle (GV) precede germinal vesicle breakdown (GVBD), enabling the resumption of meiosis—a phenomenon widely documented across diverse animal models [1–3]. Initially, chromatin within the GV exhibits a diffuse configuration; however, as oocyte development progresses, it undergoes condensation into distinct structures. This transformation signifies the acquisition of maturation competence and the potential for further developmental progression.

Research on various animal species, including humans [4, 5], monkeys [6], mice [7–10], rats [11, 12], horses [13, 14], goats [15], pigs [1, 2], rabbits [16], and cattle [17, 18] has successively revealed that the chromatin configuration of oocytes at the GV stage exhibits distinct patterns. Moreover, a correlation between the chromatin configuration of oocytes and their developmental competence has been established. Notably, the relationship between oocyte chromatin configuration and embryonic developmental potential has been demonstrated in mice [19]. Additionally, studies have shown that changes in chromatin structure within porcine oocytes can significantly influence follicle size, thereby affecting oocyte meiosis and developmental competence [20]. In summary, the chromatin configuration of GV-stage oocytes has been identified as being associated with developmental potential and can serve as a criterion for assessing oocyte quality.

However, systematic studies on the chromatin configuration of guinea pig GV-oocytes, which share greater similarities with human reproductive physiology, are relatively scarce. Currently, in the classification of chromatin configurations in guinea pigs, the chromatin is categorized into three types based on the degree of chromatin condensation and the visibility of the nuclear membrane and nucleolus: the dispersed type (NSN), the intermediate type (SN-1), and the condensed type (SN-2). In oocytes with the NSN configuration, the chromatin is diffusely distributed throughout the nucleoplasm. In those with the SN-1 configuration, some chromatin is condensed around

the nucleolus, while the remainder is dispersed within the nucleoplasm. In oocytes with the SN-2 configuration, nearly all chromatin is condensed around the nucleolus. Previous studies have shown that oocytes retrieved from follicles with a diameter of less than 0.05 mm exhibit only the NSN of GV chromatin configuration. As follicles grow, the proportion of oocytes with the NSN configuration gradually decreases, while the proportions of oocytes with the SN-2 and GVBD configurations increase. Therefore, it can be concluded that the proportion of oocytes with the SN-2 configuration increases with follicle growth [21].

Investigations on various animal species have revealed that as follicles develop, oocytes progressively attain meiotic competence and developmental potential. However, oocytes derived from follicles of identical size continue to exhibit heterogeneity, which may impact meiotic progression, cytoplasmic maturation, and subsequent developmental capacity [1]. Therefore, it is of critical importance to precisely delineate the morphological and biological characteristics of ovarian oocytes prior to *in vitro* culture, thereby establishing criteria for assessing oocyte quality.

Research has demonstrated that the chromatin configuration of GV-oocytes is correlated with their developmental potential and transcriptional activity. In mice, oocytes with the SN (surrounded nucleolus) configuration typically exhibit transcriptional silencing dependent on pol I/pol II, while those with the NSN (non-surrounded nucleolus) configuration maintain higher levels of transcription [22, 23]. Similar observations in pigs [24], humans [25], and cattle [26] suggest that transcriptional activity ceases when chromatin begins to form perinucleolar rings. However, other studies indicate that remodeling chromatin into the SN configuration is not essential for transcriptional arrest [27]. In goats, despite the absence of heterochromatin perinucleolar rings, RNA synthesis in follicles declines sharply with follicular growth and ceases in follicles with a diameter of 3 mm [15]. In rabbits, although perinucleolar rings are formed as early as in primary follicles, transcriptional activity in oocytes does not cease in large follicles without gonadotropin stimulation [16].

Current studies on the chromatin configuration of guinea pig oocytes are not systematic, and the interspecies differences among various mammals have led to a complex classification of oocyte chromatin configurations. Moreover, research on the correlation between different chromatin configurations and developmental competence is limited. Therefore, in this study, we used guinea pigs as the experimental model and stained their GV-stage oocytes with Hoechst 33342 to investigate the changes in chromatin configuration during the growth and maturation of guinea pig oocytes, as well as the alterations in developmental competence associated with different chromatin configurations. This work aims to enhance the understanding of guinea pig oocyte chromatin at the molecular level and provide a foundation for the development of guinea pig embryo engineering.

MATERIALS AND METHODS

Experimental animals

In this study, female Dunkin Hartley guinea pigs aged 20 days and weighing 200–250 g were used as the experimental animals. The animals were purchased from Jinan Pengyue Experimental Animals Breeding Co., Ltd., Jinan, China. The guinea pigs were housed under strictly controlled lighting conditions, with a 14-h light period (06:00–20:00) and a 10-h dark period (20:00–06:00). The ambient temperature was maintained at 22 °C, and the animals had free access to food and water.

Ethical 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, Ji Nan, China (Approval No. W202111220327).

Preparation of culture medium and oocyte operation solution

All solutions used in this study, except for the culture medium, were prepared in-house. The *in vitro* operation solution for guinea pig oocytes was M2 medium, which was prepared using triple-distilled water. The live-cell fluorescent dye Hoechst 33342 (Y35467, Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China) was initially dissolved in quintuple-distilled water to a concentration of 100 μg/ml (100×) and then aliquoted and stored at −20 °C. During the experimental process, it was added to the M2 operation solution to achieve a final concentration of 10 μg/ml.

Hormonal treatment of experimental animals

Human Menopausal Gonadotropin (HMG) (Tianjin Zhongbao Pharmaceutical Co., Ltd., Tianjin, China) Treatment Protocol: Female guinea pigs aged 20 days were selected and subjected to intraperitoneal injection of HMG at a dose of 5 IU/kg at 3:00 PM daily for three consecutive days. On the fourth day, after an intraperitoneal injection of Human Chorionic Gonadotropin (HCG) (Tianjin Zhongbao Pharmaceutical Co., Ltd.) at a dose of 5 IU/kg, the animals were sacrificed at different time points. The abdominal cavity was opened, and the ovaries were removed and placed in M2 medium, where they were washed to remove blood and stripped of fat tissue.

Collection of oocytes

Female guinea pigs aged 20 days were euthanized by intraperitoneal injection of sodium pentobarbi-

tal (150–200 mg/kg). The ovaries were collected and rinsed three times with phosphate-buffered saline (PBS). Under a dissecting microscope, excess fat was removed using forceps and a surgical blade. The follicles and oocytes were then categorized into three groups based on follicle diameter (<0.5 mm, 0.5–1 mm, >1 mm) and oocyte diameter (60–69 µm, 70–79 µm, 80–89 µm). Subsequently, cumulus—oocyte complexes (COCs) were released from the follicles of different sizes into M2 medium using a syringe needle. Morphologically intact COCs were collected using a custom-made glass capillary under a stereomicroscope (AIR MP; Nikon, Tokyo, Japan).

GV chromatin configuration classification

The COCs were placed into a prepared solution of 0.1% hyaluronidase (S10060, Shanghai Yuanye Bio-Technology Co., Ltd.) to remove the cumulus cells, and the denuded oocytes were subsequently transferred to a droplet of Hoechst 33342 solution for staining in the dark for 8 min. Following staining, the oocytes were aspirated using a glass pipette and placed onto a glass slide. The corners of the slide were coated with a mixture of Vaseline and glycerol to provide support, and a coverslip was placed over the oocytes and gently pressed down. The morphology of the nucleolus and nuclear membrane was initially observed under a dissecting microscope, followed by examination of the GV chromatin configuration using an upright fluorescence microscope.

Observing the chromatin configurations of COCs during IVM

Female guinea pigs aged 20 days were selected and subjected to intraperitoneal injection of HMG at a dose of 5 IU/kg at 3:00 PM daily for three consecutive days. On the fourth day, HCG was administered intraperitoneally at a dose of 5 IU/kg. Ovaries were subsequently harvested at 0.5 h intervals, and follicles were punctured using a needle to retrieve oocytes. The oocytes were then stained with Hoechst 33342 to examine the chromatin configuration.

Detection of global RNA transcription in oocytes

COCs were labeled for 2 h in 100 µl of 199 medium (#M0393, Sigma, St. Louis, USA), containing 1 mM 5-ethyluridine (EU) at 38.5 °C in a 5% CO_2 incubator. Following EU labeling, all subsequent detection steps were performed at room temperature according to the manufacturer's instructions (Invitrogen; Click-iT RNA Imaging Kit, Oregon, USA). After removal of the cumulus cells and zona pellucida, oocytes were fixed in 4% paraformaldehyde for 40 min. After washing with PBS, membranes were permeabilized with 0.1% Triton X-100 for 30 min. Under light-protected conditions, oocytes were stained for 30 min in a solution containing 100 mM Tris (pH 8.5), 1 mM $CuSO_4$, 10–50 µM fluorescent azide, and 100 mM ascorbic acid. The oocytes were then rinsed with Click-iT reaction rinse buffer and stained with Hoechst 33342 to visualize DNA. Finally, the stained oocytes were mounted on glass slides, examined, and imaged under a fluorescence microscope (Nikon, Tokyo, Japan). Hoechst 33342 and Alexa Fluor® 488 azide were excited using a blue diode (405 nm) and an argon laser (488 nm), respectively.

Statistical analysis

For experiments involving only two experimental groups, analysis was performed using the independent-samples t-test. For experimental data with more than two groups, the data were first transformed using the least significant difference (LSD) method (percentage t-test), followed by analysis of variance (ANOVA) using the ANOVA module. In the ANOVA analysis, Duncan's multiple range test was employed to assess the significance of differences among groups. Results are presented as mean ± standard error of the mean (SEM), with $p < 0.05$ indicating statistical significance.

RESULTS

Classification of GV chromatin configurations in guinea pig oocytes

After staining with Hoechst 33342 and preparing slides for fluorescence microscopy, the chromatin configurations of GV-stage oocytes in guinea pigs were classified into five types based on the degree of chromatin condensation and distribution within the nucleus: (1) NSN (Non-Surrounded Nucleolus): The chromatin is diffusely distributed throughout the GV, with no chromatin condensation around the nucleolus. (2) pNSN (Partial Non-Surrounded Nucleolus): Chromatin begins to condense near the nucleolus, with a mixture of diffuse and condensed chromatin distributed in the nucleoplasm. (3) SN-1 (Surrounded Nucleolus – Type 1): The chromatin forms a complete ring around the nucleolus, with a mixture of diffuse and condensed chromatin distributed in the nucleoplasm. (4) cSN-1 (Condensed Surrounded Nucleolus – Type 1): The chromatin forms a complete ring around the nucleolus, with chromatin condensed into larger masses within the nucleoplasm. (5) SN-2 (Surrounded Nucleolus – Type 2): The chromatin forms a complete ring around the nucleolus, with no chromatin distributed in the nucleoplasm (Fig. 1).

Chromatin configuration in the GVs of oocytes from guinea pig follicles of different diameters

Fig. 2 and Table 1 illustrate the proportionate relationships of oocyte chromatin configurations in follicles of varying sizes. In follicles with a diameter of less than 0.5 mm, approximately 56% of oocytes exhibit the NSN chromatin configuration (55.81 ± 2.91), while about 40% display the pNSN configuration (38.51 ± 1.61). No oocytes were observed with the cSN-1 or SN-2

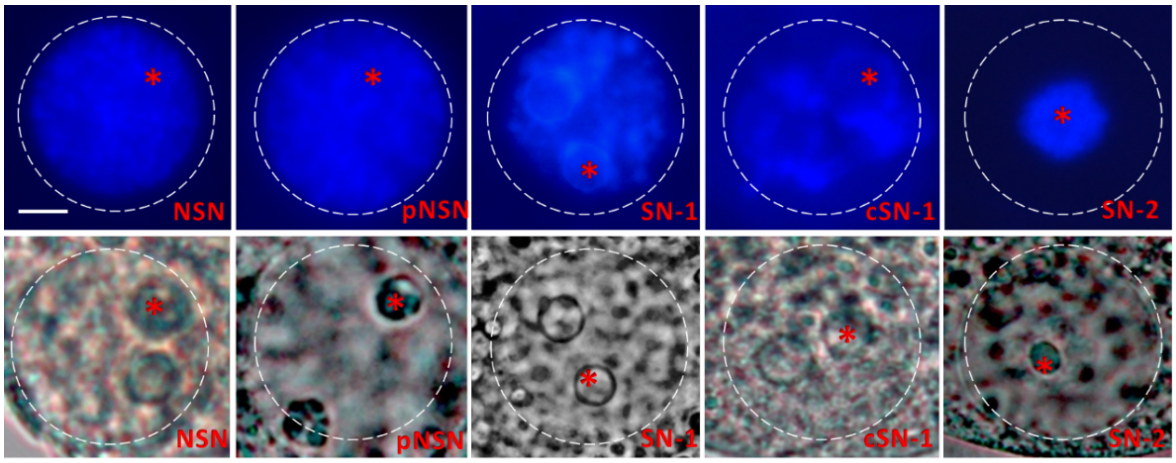

Fig. 1 Classification of GV chromatin configurations in guinea pig oocytes. (Scale bar: 20 µm). Five distinct chromatin configurations were observed, including NSN, pNSN, SN-1, cSN-1, and SN-2. The asterisk indicates the position of the nucleolus. Upper row: fluorescent staining by Hoechst 33342, Lower row: oocytes under light microscopy.

Table 1 Chromatin configuration in the GVs of oocytes from guinea pig follicles of different diameters.

Follicle diameter(mm)	No. of oocytes	Proportion of oocytes in each chromatin configuration (%)				
		NSN	pNSN	SN-1	cSN-1	SN-2
< 0.5	70	55.81 ± 2.91^{a}	38.51 ± 1.61^{a}	5.68 ± 1.33^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
0.5–1.0	84	40.46 ± 1.20^{b}	34.43 ± 1.23^{a}	17.81 ± 0.23^{b}	3.65 ± 0.38^{a}	3.65 ± 0.38^{b}
> 1.0	49	7.97 ± 1.30^{c}	18.03 ± 1.98^{b}	32.85 ± 1.43^{c}	14.52 ± 2.38^{b}	26.63 ± 1.04^{c}

There are significant differences between items with different letters in the same column ($p < 0.05$). Each treatment was replicated 3–4 times, and each replicate included approximately 30 COCs.

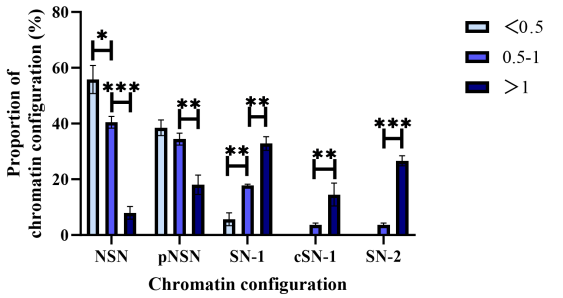

Fig. 2 The relationship between follicle size and oocyte chromatin configuration (light blue, blue, and dark blue bars represent follicles with diameters of <0.5 mm, 0.5–1 mm, and >1 mm, respectively. Asterisks (*) indicate statistical significance, with more asterisks denoting greater significance).

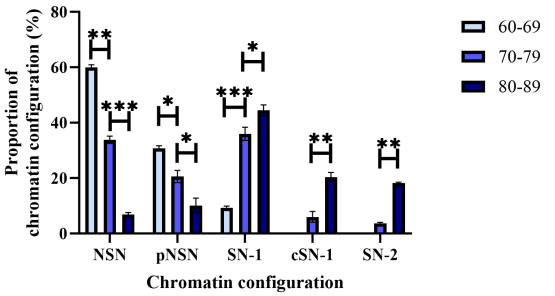

Fig. 3 The relationship between oocyte diameter and oocyte chromatin configuration (light blue, blue, and dark blue bars represent oocytes with diameters of 60–69 µm, 70–79 µm, and 80–89 µm, respectively. Asterisks (*) indicate statistical significance, with an increasing number of asterisks denoting greater significance).

configurations. In follicles with diameters ranging from 0.5–1.0 mm, the proportions of oocytes with NSN and pNSN configurations decreased (to 40.46 ± 1.20 and 34.43 ± 1.23, respectively), while the proportion of oocytes with the SN-1 configuration gradually increased (17.81 ± 0.23). The cSN-1 and SN-2 configurations began to appear, with proportions of 3.65 ± 0.38 each. In follicles larger than 1.0 mm in diameter, the proportion of oocytes with the NSN configuration significantly decreased (7.97 ± 1.30), while those with SN-1 (32.85 ± 1.43) and SN-2 (26.63 ± 1.04) configurations markedly increased.

Chromatin configuration in the GVs of guinea pig oocytes with different diameters

Fig. 3 and Table 2 depict the relationship between oocyte diameter and oocyte chromatin configurations. As shown in the figure, oocytes with a diameter of 60–69 µm predominantly exhibit the NSN chromatin configuration, accounting for approximately

Table 2 Chromatin configuration in the GVs of oocytes from guinea pig follicles of different diameters.

Diameter (μm)	Number of oocytes	Proportion of oocytes in each chromatin configuration (%)				
		NSN	pNSN	SN-1	cSN-1	SN-2
60–69	65	59.99 ± 0.51^{a}	30.75 ± 0.55^{a}	9.27 ± 0.39^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
70–79	83	33.83 ± 0.79^{b}	20.55 ± 2.19^{b}	35.99 ± 1.36^{b}	6.00 ± 1.13^{b}	3.64 ± 0.21^{b}
80–89	88	6.87 ± 0.41^{c}	10.12 ± 1.52^{c}	44.43 ± 1.16^{c}	20.39 ± 0.94^{c}	18.19 ± 0.19^{c}

There are significant differences between items with different letters in the same column ($p < 0.05$). Each treatment was replicated 3–4 times, and each replicate included approximately 30 COCs.

60% (59.99 ± 0.51). About 30% (30.75 ± 0.55) of the chromatin configurations are pNSN, with the SN-1 configuration comprising only about 10% (9.27 ± 0.39). No oocytes were observed with the cSN-1 or SN-2 configurations. In oocytes with a diameter of 70–79 μm, the proportion of oocytes with the NSN configuration decreased significantly from 60% (59.99 ± 0.51) to approximately 34% (33.83 ± 0.79), while the proportion of oocytes with the pNSN configuration decreased to about 20% (20.55 ± 2.19). The proportion of oocytes with the SN-1 configuration increased markedly (35.99 ± 1.36), and the cSN-1 and SN-2 configurations began to appear (6.00 ± 1.13 and 3.64 ± 0.21, respectively). In oocytes with a diameter of 80–89 μm, the proportions of oocytes with NSN and pNSN configurations decreased significantly to 6.87 ± 0.41 and 10.12 ± 1.52, respectively. The proportion of oocytes with the SN-1 configuration increased, albeit to a lesser extent (44.43 ± 1.16), while the proportions of oocytes with the cSN-1 and SN-2 configurations increased markedly (20.39 ± 0.94 and 18.19 ± 0.19, respectively). These results indicate that as oocyte diameter increases, the chromatin configurations of oocytes transition from predominantly NSN and pNSN to SN-1, cSN-1, and SN-2 configurations.

Changes in GV chromatin configuration during *in vivo* maturation of oocytes

Table 3 illustrates the changes in oocyte chromatin configurations during *in vivo* maturation. The results indicate that at 0 h post-HCG injection, all types of chromatin configurations were present in the oocytes, but the predominant configurations were SN-1 (33.30 ± 1.26), cSN-1 (23.56 ± 1.81), and SN-2 (29.57 ± 1.33). The proportions of NSN and pNSN configurations were relatively low (5.00 ± 1.35 and 8.57 ± 0.88, respectively), and GVBD was not observed at this time point. The NSN and pNSN configurations disappeared at 0.5 h and 1.0 h, respectively. During this period, no significant changes were observed in the proportions of SN-1, cSN-1, and SN-2 configurations. As time progressed, the proportion of oocytes with the SN-1 configuration decreased and completely disappeared by $6.0 \pm$ h. The cSN-1 and SN-2 configurations showed an initial increase followed by a decrease, with the SN-2 configuration declining at 5.5 h post-HCG injection, although this decrease was not significant. GVBD began to appear at 1.0 h and gradually increased thereafter (66.38 ± 1.13). These findings suggest that the SN-2 configuration represents a more mature chromatin state, and GVBD occurs following oocyte maturation.

Relationship between chromatin configuration and transcriptional activity in guinea pig oocytes

Fig. 4 presents the RNA transcription activity in guinea pig oocytes with different GV chromatin configurations. Each vertical column represents the same oocyte, with the upper panel showing the Hoechst 33342 staining image and the lower panel displaying the EU (5-Ethynyluridine) staining image in green. Transcriptional activity was detected in oocytes with NSN, pNSN, SN-1, and cSN-1 chromatin configurations, while no RNA transcription activity was observed in oocytes with the SN-2 configuration.

To investigate the relationship between chromatin configuration and transcriptional activity, RNA transcription was examined in oocytes with different chromatin configurations using 5-Ethynyluridine (5-EU) labeling. As shown in Table 4, among all the oocytes analyzed, 100% of oocytes with NSN and pNSN configurations exhibited RNA transcriptional activity, while no transcriptional activity was detected in oocytes with the SN-2 configuration. This is because in the SN-2 configuration, oocytes have matured, that is, they no longer synthesize new RNA, so there is no transcriptional activity, but it does not mean that this oocyte does not have RNA. Transcriptional activity was observed in 55.42% of oocytes with the SN-1 configuration and 23.52% of oocytes with the cSN-1 configuration. These results confirm that in guinea pig oocytes at the GV stage, RNA transcriptional activity is negatively correlated with the degree of oocyte maturation.

DISCUSSION

In mammalian oocytes, the chromatin within the GV is initially in a diffuse state. However, as oocytes grow, the chromatin condenses into distinct configurations [8]. To date, despite the significant value of guinea pigs in the study of human diseases [28], limited reports have addressed the chromatin configurations of the GV and their potential impact on the meiotic competence of guinea pig oocytes [21]. In the present

Table 3 Changes in GV chromatin configuration during *in vivo* maturation of oocytes.

Post-HCG injection (h)	No. of oocytes	Proportion of oocytes in each chromatin configuration (%)					
		NSN	pNSN	SN-1	cSN-1	SN-2	GVBD
0	81	5.00 ± 1.35^{a}	8.57 ± 0.88^{a}	33.30 ± 1.26^{a}	23.56 ± 1.81^{a}	29.57 ± 1.33^{a}	0.00 ± 0.00
0.5	82	0.00 ± 0.00^{b}	2.30 ± 1.15^{b}	32.93 ± 0.40^{a}	29.25 ± 0.41^{b}	35.51 ± 1.21^{b}	0.00 ± 0.00
1.0	94	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	28.78 ± 0.45^{b}	28.58 ± 1.54^{b}	39.41 ± 0.98^{c}	3.23 ± 0.25
1.5	99	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	24.20 ± 1.18^{c}	23.33 ± 1.67^{bc}	37.38 ± 0.12^{cd}	15.09 ± 1.37
2.0	104	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	21.26 ± 0.50^{c}	20.03 ± 0.77^{bc}	36.59 ± 0.35^{cd}	22.12 ± 0.59
2.5	110	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	18.95 ± 0.81^{cd}	19.17 ± 0.73^{cd}	34.59 ± 0.36^{d}	27.29 ± 0.50
3.0	106	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	16.95 ± 1.12^{de}	16.92 ± 0.89^{de}	31.14 ± 0.62^{de}	34.99 ± 0.89
3.5	105	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	15.27 ± 1.05^{ef}	16.17 ± 0.58^{de}	26.67 ± 0.48^{fg}	41.89 ± 0.97
4.0	80	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	12.49 ± 1.17^{f}	15.05 ± 2.35^{ef}	23.74 ± 1.12^{gh}	48.72 ± 1.67
4.5	76	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	10.45 ± 0.72^{g}	14.44 ± 0.51^{fg}	21.13 ± 1.02^{hi}	53.99 ± 0.29
5.0	63	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	7.95 ± 1.62^{gh}	12.64 ± 1.33^{fg}	20.66 ± 1.66^{hi}	58.74 ± 0.84
5.5	65	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	5.98 ± 0.86^{hi}	11.07 ± 2.17^{g}	19.71 ± 2.43^{i}	63.23 ± 1.00
6.0	54	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{i}	7.60 ± 1.91^{g}	26.02 ± 1.94^{j}	66.38 ± 1.13

GVBD: germinal vesicle breakdown. a–e: There are significant differences between items with different letters in the same column ($p < 0.05$). Each treatment was replicated 3–4 times, and each replicate included approximately 30 COCs.

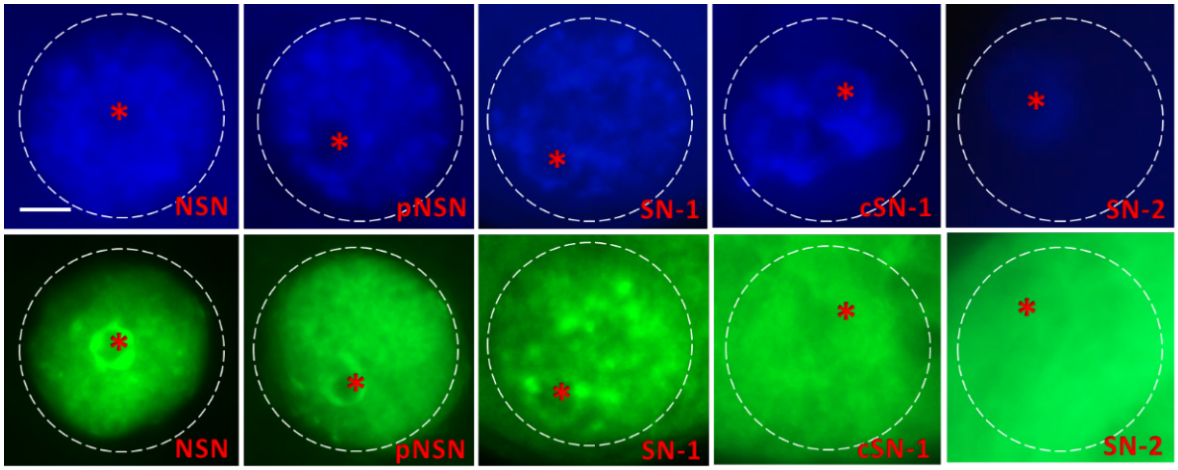

Fig. 4 Transcriptional activity in oocytes of different configurations in guinea pigs. Scale bar = 20 µm. * indicates the position of the nucleolus. Upper row: fluorescent Hoechst 33342 staining, Lower row: 5-Ethynyluridine staining.

study, we stained guinea pig oocytes with Hoechst 33342 and classified the chromatin configurations of the GV stage into NSN, pNSN, SN-1, cSN-1, and SN-2 configurations based on the distribution and degree of condensation of chromatin within the nucleoplasm.

In most mammals (with the exception of goats), as oocytes grow, the chromatin within the GV condenses from a diffuse state into distinct configurations [2, 12, 15, 16]. When the distribution of GV chromatin configurations in guinea pig oocytes derived from follicles of different sizes and oocytes with varying diameters were compared, we found that as oocytes grow and mature *in vivo*, the GV chromatin condenses progressively, transitioning from the NSN configuration to the SN-2 configuration, with a distinct perinuclear ring visible around the nucleolus. During *in vivo* matura-

Table 4 Relationship between chromatin configuration and transcriptional activity in guinea pig oocytes.

Configuration	Oocytes with transcriptional activity	Total	% Transcription
NSN	86	86	100.00 ± 0.00
pNSN	72	72	100.00 ± 0.00
SN-1	71	128	55.42 ± 0.89
cSN-1	33	141	23.52 ± 1.55
SN-2	0	63	0.00 ± 0.00

tion, oocytes with the NSN and SN-1 configurations gradually transform into the SN-2 configuration. This is consistent with observations in other animals, such as mice [16, 25, 29], pigs [1], and cattle [30], where chromatin undergoes a transformation before the occurrence of GVBD, and then synchronously adopts the same configuration.

During oogenesis, transcriptional activity is highly active to accumulate large amounts of maternal RNA in preparation for early embryonic development [27]. In this work, we investigated the relationship between chromatin configuration and transcriptional activity in guinea pig oocytes. The results showed that 100% of oocytes with NSN and pNSN configurations exhibited RNA transcriptional activity, while no transcriptional activity was detected in oocytes with the SN-2 configuration, as similarly reported in mice [22]. Transcriptional activity was observed in 55.42% of oocytes with the SN-1 configuration and 23.52% of oocytes with the cSN-1 configuration. Similar to our findings, studies have reported the relationship between transcriptional activity and chromatin configuration in oocytes of mice [3, 10], pigs [1] and goats [31]. Moreover, the larger the diameter of human/mice oocytes, the weaker the transcriptional activity, which is due to the gradual change in the chromatin configuration with the increase in oocyte diameter [25]. Our results in guinea pig oocytes show similar relation, the more mature the oocytes, the weaker the transcriptional activity. Although there are three chromatin configurations in human oocytes, we classified the chromatin configurations in more detail in guinea pigs in this study. We verified the five types of chromatin configuration in forming oocytes according to our classification by staining live cells. This study on guinea pigs oocytes provides criteria for oocyte selection, which is valuable in the context of human embryo engineering and assisted reproductive technology.

CONCLUSION

In summary, the present study has elucidated the relationship between chromatin configuration and developmental competence in guinea pig oocytes under *in vitro* conditions. These observations provide valuable evidence for the selection of high-quality oocytes and may thus be beneficial for embryo engineering involving guinea pigs.

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