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Chromatin configurations in the germinal vesicle of mammalian oocytes

Jing-He Tan[1], Hui-Li Wang, Xing-Shen Sun, Yong Liu, Hong-Shu Sui, and Jie Zhang

Laboratory for Animal Reproduction and Embryology, College of Animal Science and Veterinary Medicine, Shandong Agricultural University, Tai-an City, Shandong Province 271018, Peolple's Republic of China

[1]Correspondence address. Fax: +86-538-8241419; E-mail: tanjh@sdau.edu.cn

ABSTRACT: In all the studied mammalian species, chromatin in the germinal vesicle (GV) is initially decondensed with the nucleolus not surrounded by heterochromatin (the NSN configuration). During oocyte growth, the GV chromatin condenses into perinucleolar rings (the SN configuration) or other corresponding configurations with or without the perinucleolar rings, depending on species. During oocyte maturation, the GV chromatin is synchronized in a less condensed state before germinal vesicle breakdown (GVBD) in species that has been minutely studied. Oocytes may also take on a SN/corresponding configuration during early atresia, but they undergo GVBD at the advanced stage of atresia. As not all the species show the SN configuration while in all the species, gene transcription always stops at the late stage of oocyte growth, it is suggested that not the formation of perinucleolar rings but a thorough condensation of GV chromatin is essential for transcriptional repression. The GV chromatin configuration is highly correlated with oocyte competence; oocytes must end the NSN configuration before they gain the full meiotic competence, and they must take on the SN/corresponding configurations and stop gene transcription before they acquire the competence for early embryonic development. While factors inhibiting follicle atresia tend to synchronize oocytes in a chromatin configuration toward maturation, factors inducing follicle atresia tend to synchronize oocytes in a chromatin configuration reminiscent of early atresia. Furthermore, although condensation of GV chromatin is associated with transcriptional repression, both processes may not be associated with histone deacetylation during oocyte growth.

Key words: chromatin configuration / gene transcription / developmental competence / oocyte / mammals

Introduction

Mammalian oocytes remain at the diplotene stage of the first meiosis during development from fetal life until puberty when the LH surge stimulates the resumption of meiosis. The nucleus of an oocyte arrested at the diplotene stage is traditionally termed a germinal vesicle (GV). During mammalian oocyte growth, the GV is subject to several levels of chromatin modifications and remodeling for the control of gene expression. These include not only the local chromatin modifications at specific promoter regions and *cis*-acting regulatory elements of single copy genes but also the large-scale chromatin remodeling throughout large sections of the genome (De La Fuente, 2006; Lodde *et al*., 2007). Since the large-scale remodeling of chromatin is manifested as dynamic changes in GV chromatin configurations, and it is critical to confer the oocyte with meiotic as well as developmental competence, GV chromatin configurations during oocyte growth and maturation have been a subject of considerable interest in recent years. The objective of this paper is to review studies, including our own, on oocyte GV chromatin configurations of different species.

Classification of oocyte GV chromatin configurations in different species

The GV oocytes from antral follicles of mice were grouped into two classes according to chromatin distribution in the nucleus (Mattson and Albertini, 1990; Wickramasinghe *et al*., 1991; Debey *et al*., 1993; Zuccotti *et al*., 1995). In the first class, chromatin was rather condensed and was particularly confined around the nucleolus, termed the surrounded nucleolus (SN) configuration (Fig. 1B). In the second class, chromatin was less condensed and was not confined around the nucleolus, termed the non-surrounded nucleolus (NSN) configuration (Fig. 1A). Intermediate configurations included one with aggregates of heterochromatin apposed to the nucleolus (partly NSN) and one with partial perinucleolar ring of heterochromatin (partly SN). Rat oocytes were similar to the mouse oocytes in classification of GV chromatin configurations (Zhang *et al*., 2008). In pig oocytes, the diffuse chromatin (GV0) condensed successively into GV1, GV2, GV3 and GV4 conformations. While the GV0 was similar to the NSN in mouse oocytes, chromatin condensed into a

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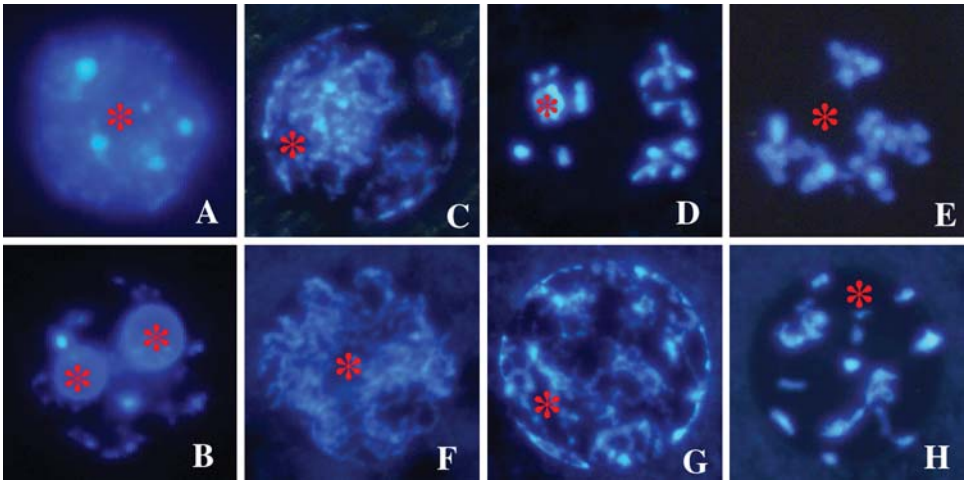

Figure 1 Photographs showing germinal vesicle (GV) chromatin configurations from the complete SN (mouse), partial SN (cattle) and non-SN (goat) species. For definition of the complete SN, partial SN and non-SN species please refer to the text in the Conclusion section. The photos were taken under a fluorescence microscope at a magnification of ×400, following Hoechst 33 342 staining. The position of the nucleolus is indicated by asterisks. (**A** and **B**) show mouse germinal vesicles with NSN and SN configurations, respectively. (**C**–**E**) show bovine germinal vesicles with NSN, SN and C configurations, respectively. (**F**–**H**) show caprine germinal vesicles with GV1 (NSN), GV2n and GV3c configurations, respectively. It should be noted that in the (C) configurations of bovine oocytes (E) as well as in the GVn (G) and GVc (H) configurations of goat oocytes, nucleoli are not surrounded with heterochromatin although the chromatin has condensed into heterochromatin network or clumps.

ring or horseshoe around the nucleolus in all other patterns (Motlik and Fulka 1976; Guthrie and Garrett, 2000; Sun *et al*., 2004). In monkeys, while the GV1 oocytes exhibited a NSN pattern, the GV2 and GV3 oocytes were distinguished by a partial or complete rim of condensed chromatin (CC) at the nucleolar surface (Lefevre *et al*., 1989; Schramm *et al*., 1993). The GV chromatin of rabbit oocytes was classified into NSN and SN configurations, and the SN chromatin was further condensed into four subclasses (Wang *et al*., 2008): the netlike (NL) configuration with netlike chromatin, the loosely condensed (LC) pattern with small clumps of chromatin, the tightly condensed (TC) configuration with larger clumps of chromatin and the singly condensed (SC) pattern with a single large clump of chromatin. Rabbit oocytes were more similar to human oocytes than oocytes from other species in GV chromatin configurations. Thus, the A, B, C and D patterns of GV chromatin in human oocytes (Combelles *et al*., 2002) looked similar, respectively, to the NL, SC, TC and LC configurations of rabbit oocytes (Wang *et al*., 2008).

The GV chromatin configurations in the horse included (i) fibrillar, having strands of chromatin throughout the GV; (ii) intermediate, having strands or irregular masses of chromatin over half the GV; (iii) loosely (LCC) and (iv) tightly condensed chromatin (TCC), having chromatin in one irregular or circular mass, usually surrounding a nucleolus and (v) fluorescent nucleus, having homogeneous chromatin in the entire GV (Hinrichs *et al*., 2005). The dog is similar to the horse in GV chromatin configurations, with its GV1, GV2 and GV4/5 roughly corresponding to the fibrillar/intermediate, LCC and TCC in the horse, respectively (Lee *et al*., 2008). The GV chromatin of bovine oocytes was classified into five configurations: NSN with diffuse chromatin in the whole GV (Fig. 1C), N with netlike chromatin, C with clumped chromatin (Fig. 1E), SN with clumped chromatin and surrounded nucleoli (Fig. 1D) and F with floccular chromatin near the nucleoli and nuclear envelope (Liu *et al*., 2006). Among these bovine configurations, only the SN had nucleolus surrounded with heterochromatin. Three GV chromatin configurations were observed in sheep oocytes: NSN and SN were similar to those described in other species, while the SNE pattern had CC near both the nucleolus and nuclear envelope (Russo *et al*., 2007). Based on both the size of nucleoli and the degree of chromatin condensation, the GV chromatin of goat oocytes was classified into GV1 (NSN) with large nucleoli and diffuse chromatin (Fig. 1F), GV2 with medium-sized nucleoli and condensed netlike (GV2n, Fig. 1G) or clumped (GV2c) chromatin, GV3 with small nucleoli and netlike (GV3n) or clumped (GV3c, Fig. 1H) chromatin and GV4 with no nucleolus but clumped chromatin (Sui *et al*., 2005). The GV chromatin in the goat never condensed into a perinucleolar ring. Chromatin configurations in different species have been related with the SN and NSN configurations of mouse oocytes in Table I.

Changes in GV chromatin configurations during oocyte growth and maturation

The distribution of GV chromatin configurations during oocyte growth and maturation in different species is summarized in Table II. In mice, only the NSN configuration is found in pre-antral follicles (Mattson and Albertini, 1990; Debey *et al*., 1993), and the SN configuration does not appear until antral follicles around Day 18 after birth (Wickramasinghe *et al*., 1991). In addition, the SN oocytes are larger in size (Wickramasinghe *et al*., 1991; Debey *et al*., 1993; Zuccotti *et al*., 1995) and show more pronounced M-phase characteristics than the NSN oocytes (Wickramasinghe *et al*., 1991). In the pig, the GV1 configurations appeared in early antral follicles and increased as the GV0

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Table I Connections between the NSN or SN configurations of the mouse and the corresponding chromatin configurations of other species

Species	NSN/corresponding configurations	SN/corresponding configurations	References
Mouse	NSN	SN	Mattson and Albertini (1990), Zuccotti *et al.* (1995)
Pig	GV0	GV1, GV2, GV3	Sun *et al.* (2004)
Monkey	GV1	GV2, GV3	Schramm *et al.* (1993)
Human	NSN	A, B, C, D	Miyara *et al.* (2003), Combelles *et al.* (2002)
Rabbit	NSN	NL, LC, TC, SC	Wang *et al.* (2008)
Cattle	NSN	N (GV1), C (GV2), SN (GV3)	Liu *et al.* (2006)
Sheep	NSN	SN, SNE	Russo *et al.* (2007)
Goat	GV1	GVn, GVc	Sui *et al.* (2005)
Horse	Fibrillar, Intermediate	LCC, TCC	Hinrichs *et al.* (2005)

For full names and definitions of abbreviations used in this table please refer to the text in the section 'Classification of oocyte GV chromatin configurations in different species'.

patterns disappeared; by 4-h post-hCG injection, over 90% oocytes were with GV1 configurations; after that, GV1 decreased as germinal vesicle breakdown (GVBD) increased (Nagai *et al.*, 1997; Guthrie and Garrett, 2000; Sun *et al.*, 2004). In antral follicles of monkeys, while the GV1 decreased, the GV3 increased with follicular growth, and the percentage of GV3 oocytes decreased by 48 h of culture, with a corresponding increase in metaphase II oocytes (Schramm *et al.*, 1993). Human GV oocytes showed size-dependent changes in chromatin configuration that followed those observed in mouse GV oocytes (Miyara *et al.*, 2003). In rabbits, about half of the oocytes in primordial follicles had a NSN configuration whereas the other half had a SN configuration. Chromatin of SN oocytes further condensed with follicular growth, and thus, NL, LC and TC were prevalent in small, medium and large antral follicles, respectively (Wang *et al.*, 2008). Rabbit oocytes were synchronized in the LC configuration before GVBD following *in vivo* treatment with gonadotrophin.

All the bovine oocytes were at the NSN stage in the <0.7 mm antral follicles, but the NSN pattern disappeared in 1.5 mm follicles when the N, C and SN patterns began to emerge (Liu *et al.*, 2006). The number of N, C and SN oocytes increased with continued growth, and were synchronized in the F configuration before GVBD during maturation *in vivo*. In sheep, all the oocytes from pre-antral follicles displayed a NSN configuration; the SN pattern began to emerge in early antral follicles; and oocytes from medium and pre-ovulatory

Table II Distribution of GV chromatin configurations during follicle or oocyte growth and maturation in different mammalian species

Species	Pre-antral	Small antral	Medium antral	Large antral	Prior to GVBD	Refer.
Mouse	NSN (91–100%)	SN (84%)	SN (100%)	SN (100%)		Mattson and Albertini (1990)
	NSN (100%, 10–40 μm oocyte)	NSN (86–95%, 40–60 μm oocyte)	NSN (66%, 60–70 μm oocyte)	SN (53%, 70–80 μm oocyte)		Zuccotti *et al.* (1995)
Pig		GV0 (>73%, <1-mm follicle	GV1, GV3 (30%, 43%, 2–3-mm follicle)	GV1 (67%, 3–6-mm follicle)	GV1 (92%, 4-h post-hCG)	Sun *et al.* (2004)
Monkey	GV1	GV2, GV3	GV2, GV3	GV2, GV3		Schramm *et al.* (1993)
Human		A (105-μm oocyte)	B (112-μm oocyte)	C (117-μm oocyte)		Miyara *et al.* (2003)
Rabbit	NSN, NL (47%, 53%, Primordial follicle)	NL (100%, <0.5-mm follicle)	LC (78%, 0.7–1-mm follicle)	TC (>65%, >1-mm follicle)	LC (78%, 2.5-h post-hCG)	Wang *et al.* (2008)
Cattle		NSN (100%, <0.7-mm follicle)	N, C, SN (22–45%, 1.5–6-mm follicle)	N, C, SN (23, 21, 55%, >6-mm follicle)	F (63%, 51-h post-PG)	Liu *et al.* (2006)
Sheep	NSN (100%)	SN (67%, 0.4-mm follicle)	SNE (3-mm follicle)	SNE (>6-mm follicle)		Russo *et al.* (2007)
Goat	GV1 (100%)	GV1(100%, 0.5–0.8-mm follicle)	GV2n, GV2c, GV3n, GV3c (all about 20%, 1–3-mm follicle)	GV3n, GV3c (59%, 20%, >3-mm follicle)	GV3n (92%, 4-h post-hCG)	Sui *et al.* (2005)

For full names and definitions of abbreviations used in this table please refer to the text in the section 'Classification of oocyte GV chromatin configurations in different species'.

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follicles displayed the SNE pattern (Russo *et al*., 2007). In the horse, the proportion of oocytes having CC configurations increased with increasing follicle size (Hinrichs and Schmidt, 2000). In the dog, more GV2 oocytes were observed in the anestrous phase than in the follicular or luteal phases, or in ovulated oocytes, while more GV5 oocytes were in ovulated groups than in the other phases (Lee *et al*., 2008). In goats, all the oocytes from pre-antral and small antral follicles were at the GV1 stage and while the proportion of GV1 and GV2 oocytes decreased, that of GV3 oocytes increased with follicular growth (Sui *et al*., 2005). During maturation *in vivo*, all the GV1 and GV2 patterns turned into GV3, and most of the GV3c turned into GV3n before GVBD.

Correlation between GV chromatin configuration and transcriptional activity of oocytes

In mice, SN- and partial SN-type oocytes were silent in relation to pol I- and pol II-dependent transcription, while NSN-type oocytes were actively transcribing (Bouniol-Baly *et al*., 1999; De La Fuente and Eppig 2001). Transcriptional repression in fully grown mouse oocytes with the SN configuration correlated with higher rates of meiotic maturation (Liu and Aoki, 2002). In pig oocytes, RNA synthesis decreased significantly from 2-mm follicles and stopped completely in follicles larger than 3 mm in diameter (Motlik *et al*., 1984a; Bjerregaard *et al*., 2004). The GV1 (SN) configuration became dominant in 2-mm porcine follicles (Sun *et al*., 2004). In humans, while smaller oocytes with an A configuration displayed a strong transcriptional activity, larger oocytes with a C configuration ended RNA transcription (Miyara *et al*., 2003). Human oocytes from antral follicles with NSN-like characteristics showed a marked [^{3}H]uridine incorporation, while those forming a karyosphere (SN) stopped nuclear transcription (Parfenov *et al*., 1989). In cattle, RNA synthesis decreased with oocyte growth (Fair *et al*., 1995, 1996), and it was ended when oocytes assumed a SN (GV3) configuration (Lodde *et al*., 2008).

Although the above results suggest that the transcriptional activity seems to stop as soon as CC begins to wrap around the nucleolus, other studies indicate that remodeling chromatin into the SN configuration is not strictly required for global transcriptional repression. In goats, while a perinucleolar ring of heterochromatin never formed, RNA synthesis of oocytes decreased dramatically with follicular growth (De Smedt *et al*., 1994) and ceased in 3-mm follicles (Sui *et al*., 2005). In rabbits, although a perinucleolar ring was formed as early as in primary follicles, the transcriptional activity of oocytes did not cease in the largest follicles if not stimulated with gonadotrophins (Sutovský *et al*., 1993; Wang *et al*., 2008). Transcriptional activity was also described in human pre-ovulatory oocytes (Tesarik *et al*., 1984). In addition, although the transition into the SN configuration failed to occur in pre-ovulatory oocytes obtained from gonadotrophin-stimulated mice deficient in nucleoplasmin 2 (Npm2$^{-/-}$), global transcriptional activity was still repressed in these oocytes (De La Fuente *et al*., 2004). The transcriptional activity in goat oocytes was associated with only the GV1 or GVn but not the GVc configurations, and that of rabbit oocytes was with only the NL or LC but not the TC or SC configurations. It should be noted that quite a lot of chromatin remained decondensed over the nucleoplasm in rabbit NL oocytes which had nucleolus surrounded with heterochromatin but were actively transcribing (Wang *et al*., 2008).

Correlation between GV chromatin configuration and the acquisition of developmental competence of oocytes

In mice, the percentage of oocytes that did not resume maturation was significantly higher and the meiotic progression was slower in the NSN than in the SN class (Wickramasinghe *et al*., 1991; Debey *et al*., 1993; Liu and Aoki, 2002). When fertilized and further cultured, none of the metaphase II NSN oocytes developed beyond the 2-cell stage while 18% of the metaphase II SN oocytes developed to blastocyst (Zuccotti *et al*., 2002). In pigs, the ability of oocytes to complete meiotic maturation was acquired in antral follicles of about 2 mm in diameter (Motlik *et al*., 1984b) and the GV0 configuration of chromatin disappeared completely in follicles of about the same size (Sun *et al*., 2004). In monkeys, while many GV3 oocytes developed to the metaphase II stage, GV1 and GV2 oocytes did not progress in GV status by 48 h of culture (Lefevre *et al*., 1989; Schramm *et al*., 1993). In humans, only oocytes with a C pattern of GV chromatin can resume meiosis among the four configurations described (Combelles *et al*., 2002). Rabbit oocytes with the NL configuration are meiosis-incompetent, whereas those with LC or TC are fully meiosis-competent; oocytes with a TC configuration are more competent in blastulation than oocytes with a LC configuration (Wang *et al*., 2008).

In the horse, oocytes with fibrillar chromatin showed a significantly lower rate of maturation than did oocytes in the LCC or TCC configurations (Hinrichs *et al*., 2005). In the dog, a greater percentage of ovulated oocytes progressed to MII *in vitro* than oocytes collected from ovaries (Lee *et al*., 2008). Bovine oocytes gained the ability to complete MII in 1.8–3 mm follicles (Motlik and Fulka, 1986), and the NSN pattern disappeared completely in follicles larger than 1.5 mm (Liu *et al*., 2006). While only 6% of the GV0 (NSN) bovine oocytes were able to complete the first meiotic division, the majority of GV1 (N), GV2 (C) and GV3 (SN) oocytes reached MII stage, and a significantly higher percentage of GV2 and GV3 oocytes reached the blastocyst stage than GV1 oocytes (Lodde *et al*., 2007). The low meiotic competence of fetal bovine oocytes has been attributed to their being at earlier stages of GV development than cow oocytes (Chohan *et al*., 2003). Goat oocytes became MII-competent in 1.5 mm follicles (De Smedt *et al*., 1994; Crozet *et al*., 1995; Ma *et al*., 2003) and the percentage of GV1 oocytes decreased to 16% in 1–1.8 mm follicles (Sui *et al*., 2005). Goat oocytes gain the ability to blastulate in follicles larger than 2 mm in diameter (Han *et al*., 2006) when most of them took on a GV3n or GV3c configuration (Sui *et al*., 2005). The relationship between chromatin configuration, transcriptional activity

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and developmental competence of oocytes in different species has been summarized in Table III.

The effect of follicular atresia on oocyte GV chromatin configurations

Although observations during follicular growth suggest that the SN or its corresponding configurations observed at the late stage of oocyte growth represent a stage of GV oocyte that is more advanced toward ovulation, the possibility that these configurations could represent a step toward atresia has been considered. In fact, several studies have shown that the SN/corresponding configurations represent degeneration toward atresia. In mice, while the NSN and SN were equal in number in breeding females, 88% of the oocytes showed a SN configuration in aged females (Zuccotti *et al.*, 1995). In monkeys, while the frequency of GV1 oocytes was higher, that of GV3 oocytes was lower in healthy than in atretic follicles, and GVBD was frequent in oocytes from atretic follicles (Lefevre *et al.*, 1989). In humans, 27 of the 44 oocytes examined showed karyospheres (GV3) (Parfenov *et al.*, 1989). Since the atretic follicles are predominant in antral follicles of adult ovaries (Pimenova and Nikitin, 1979; Nikitin *et al.*, 1982), most of the human oocytes examined might have been atretic. In equine oocytes, chromatin condensation to the CC configuration occurred not only during growth of the pre-ovulatory follicle but also during follicular atresia (Hinrichs, 1997).

However, other studies did not confirm any tendency of SN/corresponding configurations toward atretic follicles. In pigs (Guthrie and Garrett, 2000; Sun *et al.*, 2004) and goats (Sui *et al.*, 2005), no significant difference in distribution of chromatin configurations was observed between healthy and atretic follicles. However, the proportion of oocytes undergoing GVBD increased significantly in the atretic follicles in both species. In rabbits, while the proportion of TC configuration did not differ between non-atretic and atretic follicles, the number of oocytes with SC or GVBD increased significantly in the atretic follicles (Wang *et al.*, 2008). In cattle, proportions of C and SN patterns were significantly higher in non-atretic than in atretic oocytes, with most of the atretic oocytes at F and GVBD stage (Liu *et al.*, 2006). It should be noted that the follicles classified as atretic in these studies were all at the late atretic stage with apparent atretic signs, suggesting that the follicles classified as non-atretic must contain some at the early atretic stage. In rabbits, the number of TC oocytes increased sharply in the large 'non-atretic' follicles (Wang *et al.*, 2008). These large follicles could be pre-ovulatory follicles that failed to ovulate in the absence of a coital stimulus and hence at the early atretic stage with no obvious signs of atresia. Rabbit oocytes isolated from the large follicles were often found with expanded cumuli, as were the horse oocytes from atretic follicles (Hinrichs and Williams, 1997). Furthermore, rabbit oocytes with a TC configuration showed a

Table III Timing for some cellular events during growth and maturation of mammalian oocytes

Species	SN/correspond. configurations[a]	Cessation of RNA synthesis	Meiotic competence	Blastulation competence
Mouse	Early antral follicles, Dpb 17–18 (Bouniol-Baly *et al.*, 1999; Mattson and Albertini, 1990)	SN (Bouniol-Baly *et al.*, 1999; De La Fuente and Eppig, 2001; Liu and Aoki, 2002; Miyara *et al.*, 2003)	Few NSN, most SN (Zuccotti *et al.*, 2002)	SN (Zuccotti *et al.*, 2002)
Pig	GV1, 2-mm follicles (Sun *et al.*, 2004)	3-mm follicles (Bjerregaard *et al.*, 2004)	1–3-mm (Wu *et al.*, 2006) or 2-mm (Motlik *et al.*, 1984b) follicles	1–3-mm follicles (Wu *et al.*, 2006)
Human	B configuration, antral follicles, 112-μm oocyte (Miyara *et al.*, 2003)	C configuration, 118-μm oocyte (Miyara *et al.*, 2003)	C configuration (Combelles *et al.*, 2002)	
Monkey	GV3, 0.5–0.7-mm antral follicles (Schramm *et al.*, 1993)		GV3 (Schramm *et al.*, 1993)	
Rabbit	NL, primary follicles (Wang *et al.*, 2008)	TC (Wang *et al.*, 2008)	LC (Wang *et al.*, 2008)	TC (Wang *et al.*, 2008)
Horse			Intermediate (Hinrichs *et al.*, 2005)	
Dog			>2-mm follicle (Songsasen and Wildt, 2005)	
Cattle	SN, N and C, 1.5-mm follicles (Liu *et al.*, 2006)	GV3 (SN), 122-μm oocytes (Lodde *et al.*, 2008)	GV1 (N), 117-μm oocyte (Lodde *et al.*, 2007)	GV2 (C, SN), 119-μm oocyte (Lodde *et al.*, 2007)
Sheep	Early antral follicles (Russo *et al.*, 2007)		1-mm follicles (Ledda *et al.*, 1999)	
Goat	GVn, GVc, 1-mm follicles (Sui *et al.*, 2005)	GVc or 3-mm follicles (Sui *et al.*, 2005)	1.5-mm follicles (Ma *et al.*, 2003)	2-mm follicles (Han *et al.*, 2006)

For full names and definitions of abbreviations used in this table please refer to the text in the section 'Classification of oocyte GV chromatin configurations in different species'.
[a]Data were collected when over 60% of the oocytes taking on a specific configuration. NSN usually disappeared at the same time or shortly after the appearance of SN or corresponding configurations. Dpb, days post-birth; NA, not available.

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higher developmental potential than oocytes with a LC configuration after *in vitro* maturation (Wang *et al*., 2008). It has been reported that oocytes from early atretic follicles were more competent in development than oocytes from non-atretic follicles (Blondin and Sirard, 1995; Han *et al*., 2006; Feng *et al*., 2007).

Other factors affecting oocyte GV chromatin configurations

Gonadotrophin stimulation of follicles induced an increase in the proportion of SN oocytes in mice (Zuccotti *et al*., 1995; De La Fuente and Eppig, 2001). In rabbits, while eCG stimulation of medium follicles increased oocytes with LC configurations, treatment with porcine follicular fluid during follicular phase decreased the proportion of LC oocytes while increased TC, SC and GVBD oocytes (Wang *et al*., 2008). Injection of eCG also expedited the RNA synthesis of rabbit oocytes to an early and full completion.

In pigs (Sun *et al*., 2004) and goats (Sui *et al*., 2005), many fewer oocytes switched *in vitro* than *in vivo* from the CC configuration to patterns with less-condensed chromatin prior to GVBD. In mice, some aspects of intra-ovarian control mechanisms were abrogated during culture of oocyte–granulosa cell complexes, resulting in a higher proportion of oocytes with 'mature' chromatin than during maturation *in vivo* (De La Fuente and Eppig, 2001). In cattle, however, changes in GV chromatin configurations were similar between *in vivo* and *in vitro* maturation of oocytes (Liu *et al*., 2006). It is well known that *in vitro* production of viable porcine embryos is far lagging behind the status in cattle (Nieman and Rath, 2001). In addition, serum starvation during *in vitro* maturation tended to synchronize oocytes in chromatin configurations observed in atretic follicles in both goats (Sui *et al*., 2005) and cattle (Liu *et al*., 2006). It is known that serum starvation induces apoptosis in cultured somatic cells (Yu *et al*., 2003, 2006).

Ovary holding below body temperature tended to increase CC configurations of equine oocytes (Pedersen *et al*., 2004), and ovary holding before *in vitro* maturation induced early atretic changes, giving rise to more competent oocytes (Blondin *et al*., 1997). When bovine oocytes were *in vitro* matured after ovary transportation at a high temperature, most of them were found blocked at the SN stage without subsequent maturation (Liu *et al*., 2006). It is known that heat stress induces apoptosis of oocytes (Roth and Hansen, 2004).

There is evidence that a direct oocyte–granulosa cell communication through gap junctions may be required for a chromatin remodeling process during the final phase of oocyte growth. In cattle, for instance, the increase of chromatin condensation from GV0 to GV3 was accompanied by a higher incidence of oocyte–cumulus cells communications interruption (Lodde *et al*., 2007). The loss of gap junction-mediated communications between the germ and somatic compartment has been related with early signs of follicular atresia (Wiesen and Midgley, 1993). However, while transcriptional activity remained unabated in mouse denuded oocytes, transcriptional repression and the concomitant transition into the SN configuration occurred in >87% of cultured oocyte–granulosa cell complexes (De La Fuente and Eppig, 2001).

Correlation between epigenetic histone modifications, GV chromatin configuration and transcriptional activity of oocytes

Recent studies have demonstrated that histone modifications, such as acetylation, methylation and phosphorylation, play important roles in the regulation of chromatin structure and gene expression. In general, histone acetylation leads to the relaxation of chromatin structure and thus correlates with gene activation. In contrast, histone deacetylation leads to condensation of chromatin structure and thus correlates with gene repression (Kallin and Zhang, 2004). Therefore, the core histone tails of GV chromatin should become less acetylated as oocytes grow, because chromatin condenses and gene expression is silenced during oocyte growth. However, it was shown that fully grown mouse GV oocytes were fully acetylated at all the lysine residues on H3 and H4, but underwent deacetylation after GVBD and became acetylated again in 1-cell embryos (Kim *et al*., 2003; De La Fuente, 2006). In fact, analysis revealed that the acetylation of H3K9, H3K18, H4K5 and H4K12 increased during mouse oocyte growth and that fully-grown GV oocytes showed the most modifications (Kageyama *et al*., 2007). Furthermore, the level of histone acetylation in mouse SN oocytes was shown either similar to or even higher than that in the NSN oocytes (De La Fuente *et al*., 2004; Kageyama *et al*., 2007; Ola *et al*., 2007). Similarly, the histone H3 acetylation status was maintained, while the chromatin configuration changed from decondensed to a perinucleolar heterochromatin sheath during the growth of the pig oocytes (Bui *et al*., 2007). The inhibition of histone deacetylase (HDACs) with trichostatin A (TSA) inhibited the deacetylation of histone H3 and post-GVBD chromosome condensation in pig oocytes (Bui *et al*., 2007). Furthermore, although inhibition of HDACs with TSA induced chromatin decondensation, it did not restore transcriptional activity in mouse SN oocytes (De La Fuente *et al*., 2004). Therefore, histone acetylation may not be associated with transcriptional activation while deacetylation may not be associated with chromatin condensation during oocyte growth.

Methylation of H3K4 and H3K9 increased during growth of mouse oocytes and the fully-grown GV oocytes showed the most modifications (Kageyama *et al*., 2007). In pig oocytes, H3K9 did not become methylated until follicles developed to the antral stage (Bui *et al*., 2007). It is known that methylation of H3K4 and H3K9 is involved in the activation and suppression of gene expression, respectively (Kallin and Zhang, 2004). Thus, the increase in methylated H3K4 contradicts the decrease in transcriptional activity with oocyte growth. No phosphorylation of H3S10 or H3S28 was detected in any growing or fully-grown pig oocytes, but histone H3 phosphorylation changed in the same pattern as its deacetylation during oocyte maturation and activation (Bui *et al*., 2007). Therefore, the deacetylation of histone H3 is thought to be required for its phosphorylation in meiosis.

Conclusions

The NSN configuration of GV chromatin is found in all the studied mammalian species. Although the NSN becomes SN configuration

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in most species (the SN species), the NSN never turns into SN in goats (the non-SN species). Among the SN species, while all the NSN oocytes become SN oocytes in the complete SN species, only some of the NSN oocytes become SN oocytes in the partial SN species with the remaining assuming other condensed configurations. Therefore, in terms of the formation of perinucleolar rings, while the complete SN species (mouse, rat, rabbit, pig and primate) and the non-SN species (goat) form two extremes, the partial SN species (cattle and horse) fall in between. While the NSN chromatin condensed into SN or other corresponding condensed configurations during oocyte growth, the GV chromatin is finally synchronized in a less condensed state before GVBD in several species that have been minutely studied. Although some studies could not show any difference in the SN/corresponding configurations between non-atretic and late atretic follicles, there is evidence that oocytes are most likely take on a SN/corresponding configuration in early atretic follicles, but undergo a severe chromatin condensation or GVBD at the advanced stage of atresia. While some studies suggest a close correlation between the SN configuration and global transcriptional repression, other studies indicate that a thorough condensation of GV chromatin rather than the formation of perinucleolar rings is essential for transcriptional repression of oocytes. Oocytes must end the NSN configuration before they gain the full meiotic competence, and they must take on the SN or corresponding configurations and stop gene transcription before they acquire the competence for early embryonic development. The GV chromatin configuration is affected by various factors; while factors inhibiting follicle atresia (such as gonadotrophin) tend to synchronize oocytes in a chromatin configuration toward maturation, factors (such as thermal stress) inducing follicle atresia tend to synchronize oocytes in a chromatin configuration observed at the early atretic stage. Although condensation of GV chromatin is associated with transcriptional repression, both processes may not be associated with histone deacetylation during oocyte growth, suggesting that the regulation of chromatin condensation and gene transcription during oocyte growth might be different from that in other systems. However, many issues remain to be dealt with in this field. The criteria and nomenclature for classification of GV chromatin configurations must be unified between species and laboratories to get more correct and accurate conclusions. All the blanks in Tables II and III must be filled in to get a complete picture of GV chromatin configurations and their functions in different species. For example, the big difference in chromatin configuration between sheep and the other two ruminants (goat and cattle), and the changes in GV chromatin configuration during oocyte maturation *in vivo* (between the LH surge and GVBD) in some species, must be investigated. There are more questions than answers regarding the correlation between epigenetic histone modifications and changes in GV chromatin configuration and transcriptional activity of developing oocytes and studies on this issue will contribute to our understanding of the mechanisms and importance for chromatin remodeling.

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