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Large-scale chromatin structure and function changes during oogenesis: the interplay between oocyte and companion cumulus cells

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Abstract

The process of chromatin configuration remodeling within the mammalian oocyte nucleus or germinal vesicle (GV), which occurs towards the end of its differentiation phase before meiotic resumption, has received much attention and has been studied in several mammals. This review is aimed to highlight the relationship between changes in chromatin configurations and to both functional and structural modifications occurring in the oocyte nuclear compartment. During the extensive phase of meiotic arrest at the diplotene stage, the chromatin enclosed within the GV is subjected to several levels of regulation. Morphologically, the chromosomes lose their individuality and form a loose chromatin mass. Then the decondensed chromatin undergoes profound rearrangements during the final stages of oocyte growth in tight association with the acquisition of meiotic and developmental competence. Functionally, the discrete stages of chromatin condensation are characterized by different level of transcriptional activity, DNA methylation and covalent histone modifications. Interestingly, the program of chromatin rearrangement is not completely intrinsic to the oocyte, but follicular cells exert their regulatory actions through gap junction mediated communications and intracellular messenger dependent mechanism(s). With this in mind and since oocyte growth mostly relies on the bidirectional crosstalk with the follicular cells, experimental manipulation of large-scale chromatin configuration is discussed. Besides providing tools to determine the key cellular pathways involved in genome-wide chromatin modifications, the present findings will aid to the refinement of physiological culture systems that can have important implications in treating human infertility as well as managing breeding schemes in animal husbandry.

Keywords: chromatin, cumulus cells, gap junctions, germinal vesicle, oocyte, transcriptional activity.

Introduction

The chromatin organization and architecture is a characteristic element of the process of oocyte differentiation in mammals (Luciano and Lodde, 2013). Oocyte development is characterized by impressive changes in chromatin structure and function within the nucleus, namely the germinal vesicle (GV). These changes are crucial to confer the oocyte with meiotic and developmental competences and they occur along the process of folliculogenesis, when gamete and somatic cells communicate through junctional and paracrine mediated mechanisms (Albertini *et al.*, 2003).

Dynamic changes in GV oocyte chromatin configuration have been described in mouse (Wickramasinghe *et al.*, 1991; Debey *et al.*, 1993; Zuccotti *et al.*, 1995), rat (Mandl, 1962), human (Combelles *et al.*, 2003; Miyara *et al.*, 2003), monkey (Schramm *et al.*, 1993), horse (Hinrichs and Williams, 1997; Hinrichs and Schmidt 2000; Franciosi *et al.*, 2012), pig (Bui *et al.*, 2007; Dieci *et al.*, 2013), cattle (Fuhrer *et al.*, 1989; Chohan and Hunter, 2003; Liu *et al.*, 2006; Lodde *et al.*, 2007), buffalo (Yousaf and Chohan, 2003), goat (Sui *et al.*, 2005), sheep (Russo *et al.*, 2007), dog (Jin *et al.*, 2006; Lee *et al.*, 2008; Reynaud *et al.*, 2009), ferret (Sun *et al.*, 2009), rabbit (Wang *et al.*, 2009) and cat (Comizzoli *et al.*, 2011). Although different patterns of chromatin organization have been defined in mammals, sometimes the nomenclature can be confusing, since it is not univocal in part due to some species-specificity. For example, Surrounded Nucleolus (SN) configuration - where chromatin forms a ring around the nucleolus - has been described in the mouse as well as and in other mammals (monkey, pig, rat and human) while this configuration was not evidenced in the horse oocyte where 'fibrillar', 'intermediate' and 'condensed' configurations were documented (Franciosi *et al.*, 2012), or in the bovine, where the highest degree of chromatin compaction is found in GV3 oocytes. Moreover, very often, different acronyms were used within the same species by different authors and this made data interpretation puzzling.

Nevertheless, despite the species-specific patterning, the process of large-scale chromatin configuration changes seems to be a common process in mammals. In fact, what is clear is that the chromatin contained in the GV achieves a high degree of condensation and compaction passing through intermediate configurations, before the resumption of meiosis. Incidentally, it is worth stating that the GV3 or the SN configurations have been first described by Blackman in early 1900 in spermatocytes of millipedes

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named the 'karyosphere' (Blackman, 1903). The karyosphere "represents a transformation of meiotic chromosomes often occurring just prior to the completion of meiotic division", and a similar structure, named karyosome, exists in Drosophila (King, 1970) as well as in other phylogenetically distant organisms studied so far, suggesting a well-conserved process between species during phylogeny (Gruzova and Parfenov, 1993).

Significance of large-scale chromatin configuration changes

Differences in chromatin configuration do not only refer to morphological modifications but also to its functionality (De La Fuente, 2006; Luciano and Lodde, 2013). Several studies indicated that there is a relationship between chromatin configurations, transcriptional activity, epigenetic signature, characteristics of the ooplasm and oocyte competence and altogether these features are strictly associated one to each other. Importantly, a direct relationship between oocyte chromatin configuration and embryonic developmental competence has been ascertained in mouse (Zuccotti *et al.,* 1998, 2002) and in cow (Lodde *et al.,* 2007; Luciano *et al.,* 2011).

In growing mouse oocytes chromatin is initially decondensed in a configuration termed Non-Surrounded Nucleolus (NSN; Mattson and Albertini 1990; Debey *et al.,* 1993; Zuccotti *et al.,* 1995). With the subsequent growth and differentiation, chromatin becomes progressively condensed, forming a heterochromatin rim in close apposition with the nucleolus, acquiring a configuration termed Surrounded Nucleolus (SN; Mattson and Albertini 1990; Debey *et al.,* 1993; Zuccotti *et al.,* 1995).

The morphological variances between these two types of oocytes have a biological relevance because NSN and SN configurations have been correlated with differences in follicle size, oocyte diameter and the age of the mouse (Mattson and Albertini 1990; Zuccotti *et al.,* 1995, 1998). It has been demonstrated that the transition into the SN configuration correlates with the timely progression of meiotic maturation (Wickramasinghe *et al.,* 1991; Debey *et al.,* 1993; Zuccotti *et al.,* 1995) suggesting that SN oocytes may represent the more advanced stage of preovulatory oocytes (Mattson and Albertini 1990; Zuccotti *et al.,* 1995, 1998). Additionally, after *in vitro* maturation and fertilization, NSN oocytes are unable of development beyond the two-cell stage while SN oocytes are capable of development to the blastocyst stage (Zuccotti *et al.,* 1998, 2002). Differences in chromatin configurations have also been correlated with changes in transcriptional activity, with NSN oocytes transcriptionally active and SN oocytes associated with global repression of transcriptional activity (Bouniol-Baly *et al.,* 1999; Christians *et al.,* 1999; De La Fuente and Eppig 2001; Liu and Aoki, 2002; Miyara *et al.,* 2003).

In the cow, oocytes collected from early and middle antral follicles present four patterns of chromatin configuration (Fig. 1), from GV0 to GV3 characterized by progressive increase in condensation (Lodde *et al.,* 2007), transcriptional silencing (Lodde *et al.,* 2008; Luciano *et al.,* 2011), global DNA methylation (Lodde *et al.,* 2009) and progressive histone H4 acetylation (unpublished data), as previously reported also in mice (Akiyama *et al.,* 2004). As shown in Fig. 2, the GV0 stage shows a diffuse filamentous pattern of chromatin in the whole nuclear area; the GV1 and GV2 configurations represent early and intermediate stages, respectively, of chromatin remodeling, a process starting with the appearance of few foci of condensation in GV1 oocytes and proceeding with the formation of distinct clumps of condensed chromatin in GV2 oocytes; the GV3 is the stage where the highest level of condensation is reached with chromatin organized into a single clump (Lodde *et al.,* 2007). Importantly, oocytes with a GV0 configuration showed a very limited capacity to resume and complete meiosis I after *in vitro* maturation, while virtually all the GV1, GV2 and GV3 oocytes were able to reach MII stage, despite their GV configuration. On the contrary, only a limited percentage of GV1 oocytes reached the blastocyst stage after *in vitro* fertilization, while GV2 and GV3 oocytes showed a higher embryonic developmental potential (Lodde *et al.,* 2007).

These results further support the general principle that meiotic and developmental competencies are acquired at sequential stages of oogenesis (Albertini *et al.,* 2003), concomitantly with changes in large-scale chromatin structure (De La Fuente, 2006) and that chromatin remodeling can be considered a marker of oocyte differentiation and developmental competence.

The progressive large scale chromatin remodeling relies on functional gap-junction mediated communications between oocyte and follicular cells

During folliculogenesis oocyte growth and differentiation tightly depend on the establishment of a patent bidirectional communication between oocytes and companion granulosa cells mediated by heterologous gap junctions (Eppig, 2001; Matzuk *et al.,* 2002; Mehlmann *et al.,* 2004). In mouse, previous studies indicate that the presence of oocyte-associated granulosa cells are required for the progressive repression of transcriptional activity in fully grown oocytes (De La Fuente and Eppig, 2001) and to promote the transition from NSN to SN configuration after gonadotropin stimulation (De La Fuente and Eppig, 2001). This hypothesis is supported also by studies where gap junction mediated communications (GJC) between mouse oocyte and cumulus cells were interrupted, due to targeted deletion of the connexin 37 gene (Gja4), and chromatin condensation associated

with transcriptional repression failed to occur (Carabatsos *et al.*, 2000).

Coupling between oocyte and cumulus cells undergoes dynamic changes during follicle development and the patency of GJC between the two compartments decreases in parallel with the meiotic resumption of the oocyte (Eppig, 1982; Larsen *et al.*, 1986, 1987). However, recent studies performed in the cow, horse, dog, cat and pig (Luvoni *et al.*, 2001, 2006; Colleoni *et al.*, 2004; Luciano *et al.*, 2004; Dieci *et al.*, 2013) indicated that morphologically healthy oocyte-cumulus cells complexes isolated from antral follicles without evident signs of atresia form a heterogeneous population characterized by different degree of GJC functionality.

In the cow, the direct oocyte-granulosa cell communication through gap junctions seems a requisite for chromatin remodeling during the final phase of oocyte growth (Lodde *et al.*, 2007; Luciano *et al.*, 2011). This is supported by the evidences that, at the time of collection, the pattern of uncondensed chromatin in GV0 oocytes is associated with fully open GJC. On the contrary, the percentage of oocytes with functionally open communications significantly decreases with the increase of chromatin condensation, from GV1 to GV3 oocytes (Lodde *et al.*, 2007; Luciano *et al.*, 2011), indicating that when oocytes reach the highest level of chromatin condensation, there is a greater probability of loosing coupling with follicular cells (Lodde *et al.*, 2007). On the other hand, the increase in chromatin condensation may represent a consequence of the premature interruption of the communication between the oocyte and follicular cells before final oocyte maturation, since the loss of GJC between the germ and somatic compartment has been related with early events of follicular atresia (Wiesen and Midgley, 1993).

The manipulation of GJC functionality affects chromatin configuration and transcription through cAMP-mediated mechanism(s)

The central role of GJC in the modulation of chromatin configuration, global transcriptional activity and developmental competence acquisition has been recently confirmed in bovine oocyte-cumulus cells complexes. The use of culture systems that prolonged GJC functionality sustained oocyte growth and permitted chromatin to gradually organize from GV0 to the GV1 configuration, thus allowing the oocyte to acquire the ability to mature and to be fertilized *in vitro* (Luciano *et al.*, 2011). .Yet, when GJ functionality was experimentally interrupted with the uncoupler 1-heptanol, chromatin rapidly condensed and RNA synthesis suddenly ceased. Interestingly, this effect was nullified by treatment with cilostamide, a specific inhibitor of the oocyte-specific PDE3, an enzyme-degrading cAMP (Richard *et al.*, 2001; Conti *et al.*, 2002; Sasseville *et al.*, 2009), indicating that the functional status of GJC may affect both transcriptional activity and remodeling of large-scale chromatin configuration, potentially through cAMP-dependent mechanism(s; Luciano *et al.*, 2011).

Therefore, besides the well-characterized mechanisms of action by which cAMP is known to regulate meiotic resumption (Downs, 2010; reviewed in Bilodeau-Goeseels, 2011), these studies may suggest that cAMP could be also involved in controlling the activity of factors that modulate transcription and large-scale chromatin remodeling during the final phase of oocyte growth and before the resumption of meiosis. In fact, since the preservation of a proper cAMP content in the oocyte even in the absence of functional GJC is able to prevent the abrupt condensation of the chromatin this makes cAMP the molecule that mostly mediates GJ action on the chromatin.

Oocyte cAMP levels are sustained by endogenous adenylate cyclases and constitutively active G-protein-coupled receptors (Mehlmann *et al.*, 2002). cAMP is generated also by cumulus cells and then transported into the oocyte through gap junctions (Anderson and Albertini 1976; Bornslaeger and Schultz, 1985). The manipulation of intracellular cAMP concentration has been demonstrated to influence functional coupling between oocyte and cumulus cells; a decrease in cAMP was accompanied by a drop in functional coupling (Luciano *et al.*, 2004; Thomas *et al.*, 2004). Several attempts have been made in order to mimic the physiological system in oocyte *in vitro* maturation taking into account the time for completing the developmental competence acquisition. These culture systems (namely pre-maturation systems) that precede *in vitro* maturation (Gilchrist and Thompson, 2007; Gilchrist, 2011; reviewed by Bilodeau-Goeseels, 2012) are based on the control of spontaneous meiosis resumption through the addition of either cAMP analogues or adenylate cyclase activator, PDE inhibitors (general or specific), or through a combination of these treatments. These treatments prevent the loss of cumulus-oocyte GJ mediated communications and increase oocyte developmental competence (Luciano *et al.*, 1999; Guixue *et al.*, 2001; Atef *et al.*, 2005; Nogueira *et al.*, 2006; Ozawa *et al.*, 2008; Shu *et al.*, 2008; Nogueira and Vanhoutte, 2009; Albuz *et al.*, 2010; Luciano *et al.*, 2011; Dieci *et al.*, 2013; Lodde *et al.*, 2013; Rose *et al.*, 2013; Zeng *et al.*, 2013; Richani *et al.*, 2014). In several systems, the maintenance of a proper cAMP concentration seems to be the main requirement to promote regular chromatin transition thus endorsing oocyte differentiation (Vanhoutte *et al.*, 2007; Luciano *et al.*, 2011; Dieci *et al.*, 2013; Lodde *et al.*, 2013).

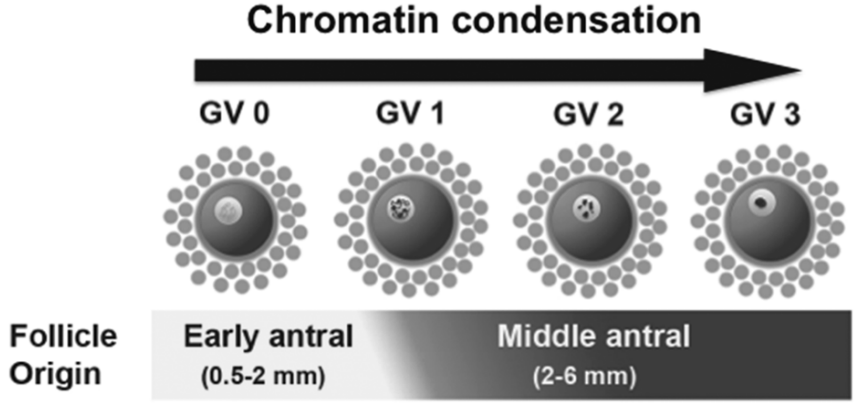
Chromatin manipulation in assisted reproduction technologies

There is no doubt that the experimental manipulation of large-scale chromatin configuration *in*

vivo and *in vitro* will provide a tool to determine the key cellular pathways and oocyte-derived factors involved in genome-wide chromatin modifications. However, assessment of large-scale chromatin configurations has also key implications in ARTs both in human and domestic mammals. It has been shown that different patterns of chromatin configuration are indicative of different metabolic properties, thus potentially representing a morphological marker to select a population of oocytes with different cultural requirements. Several studies support the notion that *in vitro* treatments aiming to improve the developmental capability of immature oocytes can have a different outcome with pre-maturation culture depending on the metabolic status of the oocyte at the time of its removal from the follicular environment (Nogueira *et al.*, 2006; Vanhoutte *et al.*, 2008, 2009). This has been confirmed also by morphological studies in the cow, which demonstrated that the pharmacological pre-maturation system can negatively affect oocytes obtained from medium antral follicles when compared with those isolated from earlier stages (Fair *et al.*, 2002).

It is of extreme importance to realize that attempts to manipulate *in vitro* large-scale chromatin configuration must be performed cautiously. In fact, even though it is true that the chromatin configuration of an oocyte is indicative of its developmental capability at the time of its collection from the follicle, pharmacological treatments forcing chromatin abruptly into a high-condensed state may not necessarily be beneficial to the oocyte competence, although fundamental in basic science-type investigation (Comizzoli *et al.*, 2011). Therefore, the design of pre-maturation strategies must take into account that chromatin condensation and spatial reorganization should occur gradually and orderly, recapitulating the process that normally occurs *in vivo*. For example, maintenance of a proper functional coupling between oocyte and cumulus seems to be crucial in sustaining an orderly chromatin condensation *in vitro* (Luciano *et al.*, 2011; Dieci *et al.*, 2013; Lodde *et al.*, 2013; Franciosi *et al.*, 2014, Reproductive and Developmental Biology Laboratory, University of Milan, Milan, Italy, unpublished data). Thus, if coupling is prematurely interrupted - i.e., when oocytes have not yet acquired full competence and are still committed to accumulating transcripts and proteins - unexpected chromatin condensation can be triggered, thus preventing proper and gradual differentiation of large-scale chromatin configuration and function.

In view of all given considerations, knowledge of the molecular mechanism(s) leading the oocyte to remodel its chromatin configuration under physiological conditions will be of great help for assisted reproductive technologies.



Transcriptional Activity	+	±	-	-	*Lodde et al, 2008*
Global 5MeC Methylation	±	+	+	+	*Lodde et al, 2009*
Acetylation AcH4 -K5 / -K12	- / ±	± / +	+/+	+/+	*Lodde et al unpublished data*
Meiotic Competence	-	+	+	+	*Lodde et al, 2007 Luciano et al, 2011*
Developmental Competence	-	±	+	+	*Lodde et al, 2007 Luciano et al, 2011 Lodde et al, 2013*

Figure 1. Transcriptional activity, global methylation, histone H4 acetylation, meiotic and developmental competence in relation to chromatin configuration in the bovine oocyte.

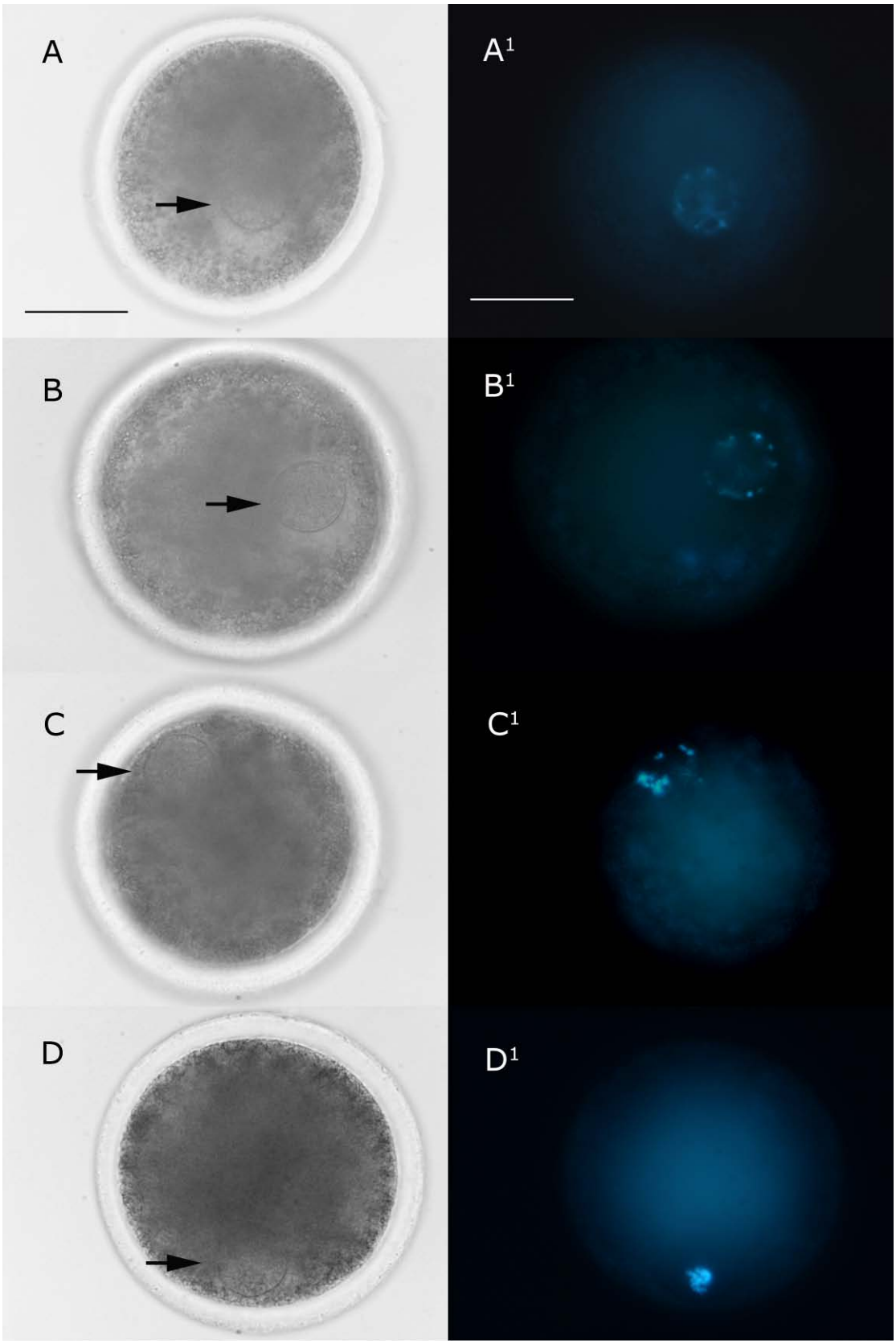


Figure 2. Bright field and fluorescent images after Hoechst 33342 labeling of bovine oocytes with GV0 (A, A1), GV1 (B, B1), GV2 (C, C1), and GV3 (D, D1) configuration (see text for stage definitions). Arrows in the bright fields indicate the nuclear envelope. Scale bar: 50 mm. From: Lodde *et al.*, 2007.

Conflict of interest

None of the authors have any conflict of interest to declare.

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