Follicular cysts in dairy cows

Naoki ISOBE

Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima-Shi, 739-8528, Japan

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Correspondence: Naoki Isobe, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima-Shi, 739-8528, Japan.
(Email: niso@hiroshima-u.ac.jp)

ABSTRACT

Follicular cysts are the most critical reproductive disorder in dairy cows and disturb normal ovarian cycle, resulting in the prolonged interval from calving to conception. Therefore, this condition causes significant economic losses to the dairy industry. Two direct causal factors of cyst are suggested in this review; the disorder of ovulation and the delay of regression. The disorder of ovulation has been accepted to be a main etiology of cystic follicle. This seems to be caused by the deficiency of positive feedback of estrogen to the hypothalamus, leading to the lack of LH surge. On the other hand, if large anovulatory follicle is regressed immediately after the failure of ovulation, its follicle does not grow more, resulting in no cystic follicle formed. Therefore, it is proposed that another cause of cystic follicle is a delay (lack) of degeneration system of follicle. This review will introduce these two causes separately referring to recent advances about follicular cyst in dairy cows.

Key words: cyst, dairy cows, estradiol, follicle, follicular regression

INTRODUCTION

Follicular cysts are the most critical reproductive disorder in dairy cows. Follicular cysts disturb normal ovarian cycle, resulting in the prolonged interval from calving to conception. Therefore, this condition can cause significant economic losses to the dairy industry.

Follicular cysts have been usually defined as anovulatory, follicular structures (25 mm in size) that persist for at least 10 days in the absence of a corpus luteum (Brown *et al*. 1982; Garverick 1997). Recently, 20 mm is also employed to be the minimum size of follicular cyst (Peter 2004; Calder *et al*. 1999, 2001).

Anovulatory cysts have been classified as follicular or luteal. Follicular cysts are usually thin-walls and secrete little progesterone and much estradiol (Fig. 1a, Table 1); luteal cysts have thicker walls and secrete much progesterone and little estradiol (Fig. 1c). Carroll *et al*. (1990) and McLeod and Williams (1991) have reported that about 42% and 54% of cysts, respectively, are luteal at some time during their lifespan. In this review, follicular cysts are mainly focused.

According to histological studies, there are two types of follicular cysts; presence (Fig. 1a) or absence (Fig. 1b) of granulosa layers (Cook *et al*. 1990; Isobe and Yoshimura 2000a,b). Cystic follicle with granulosa layers produces much amount of estradiol, whereas that without granulosa layers does not, because of the granulosa cells are main site to produce estradiol (Calder *et al*. 2001, Isobe *et al*. 2005, Table 1).

Formation of follicular cysts in dairy cows has been related to a number of environmental and hereditary factors (Garverick 1997). A direct relationship between genetics and follicular cysts is suggested by Casida and Chapman (1951), who reported

that the incidence of ovarian cysts was 26.8% in daughters of cows that had ovarian cysts, whereas daughters of cows that had no history of ovarian cysts had an incidence of ovarian cysts of 9.2%. Uterine infection and some stress also play as etiological factors in cystic follicle formation (Tanabe and Brofee 1982; Bosu and Peter 1987; Kaneene *et al*. 1987; Ribadu *et al*. 2000). Some researchers suggested that high milk production was associated with development of cystic follicle (Johnson *et al*. 1966). These various factors disturb endocrine control of cows, resulting in cystic follicle formation.

This review propose two direct causes of follicular cyst; disorder of ovulation and delay of follicular regression. It is accepted that disorder of ovulation is primary cause of follicular cyst. However, if preovulatory follicle that can not be ovulated is regressed immediately, its follicle does not grow further more, then no cystic follicle is formed. Therefore, latter secondary cause is important factor of the formation of cystic follicle in dairy cows. This article will review these two causes separately referring to recent advances about follicular cyst in dairy cows.

DISORDER OF OVULATION

It is accepted that endocrine imbalances cause lack of LH surge, leading to the anovulation of dominant follicle. Mean concentration pulse frequency and amplitude of LH were increased during the follicular phase in cows which developed cysts compared to cows which subsequently ovulated (Cook *et al*. 1991). LH pulse frequency was significantly reduced during cyst formation and persistence compared to early luteal phase and follicular phase (Ribadu *et al*. 2000). Hamilton *et al*. (1995) found that

concentrations of LH and estradiol-17β were greater in cows with cysts than in cows in ovulatory cycles around the time of ovulation or at the time when cysts reached a diameter equivalent to that of follicles that ovulated. According to Yoshioka *et al*. (1996), the plasma concentrations of estradiol-17β appeared to be high during the period of growth of the cystic structures from 20 mm to the maximum in diameter. Thus, altered LH and estradiol concentrations are suggested to be associated with the formation of cystic follicle. In the normal endocrine condition, positive feedback of estrogen stimulates hypothalamus to secrete GnRH. However, content of GnRH in the hypothalamus was lower in cows with cyst than cows without cyst (Cook *et al*. 1991). Kaneko *et al*. (2002) administered anti-estradiol antiserum to inactivate the endogenous estradiol in the cows to investigate the significant role of estradiol-feedback regulation in the formation of cyst, and observed the cystic formation of these cows. Therefore, the deficiency of positive feedback of estrogen is a primary cause of disorder of ovulation. Gümen and Wiltbank (2002) synchronized cows with an intravaginal progesterone insert (IPI) and prostaglandin F2α and induced a GnRH/LH surge with estradiol benzoate (EB) in an absence of ovulatory follicle. All cows had an LH surge and most cows developed cystic follicle like structure. Moreover, in these cows, progesterone stimulation with IPI followed by EB treatment induced LH surge and subsequent ovulation, although EB treatment without IPI did not. From these results they suggested that estradiol induction of a GnRH/LH surge requires previous exposure to progesterone and that progesterone could reinitiate responsiveness to estradiol by simply increasing functional estrogen receptor in a region of the hypothalanus. Their research strongly agrees with above-mentioned theory that deficiency of positive feedback of estrogen is primary cause of disorder of ovulation.

In the histological study, Calder *et al*. (2001) compared mRNA expression of steroidogenesis enzymes and LH and FSH receptors by in situ hybridization of dominant and cystic follicles and found that mRNAs expression of LH receptor and 3ß-hydroxysteroid dehydrogenase (3β-HSD), enzymes for conversion from pregnenolone to progesterone, was higher in granulosa cells of cysts than in dominant follicles. Isobe *et al*. (2003a) reported the localization of 3β-HSD in the cystic follicle. The frequency of 3β-HSD-positive granulosa cells in cystic follicles was significantly higher than those in the healthy follicles. The localization of P450 side chain cleavage, enzymes for conversion from cholesterol to pregnenolone, was lower in the cystic follicles than that in healthy follicles (Isobe *et al*. 2003b). These histological results suggest that the some process of steroidogenesis is altered in cystic follicles, which may be associated with disorder of feedback system between gonad and hypothalamus.

Hatler *et al*. (2003) collected blood samples from cows when follicular cysts were first diagnosed. Sixty-six percent of these cows had progesterone that fell in the intermediate range, 0.1-1.0 ng/ml (Silvia *et al*. 2002). They also investigated the fate of follicle in diameter >10 mm in the presence of cystic follicle relating to progesterone concentrations during follicular development (Hatler *et al*. 2003). In the cows having intermediate progesterone concentration, 75% of follicles formed cyst, although significantly lower percentage of follicles (41%) formed cyst in the cows with low progesterone (< 0.1 ng/mL). They proposed model for the etiology of cysts that intermediate concentration of progesterone in the circulation may cause hypothalamic insensitivity to estradiol, inducing failure of releasing a surge of GnRH. Furthermore, the phenomenon of turnover of cysts occurs because otherwise normal follicles mature and reach preovulatory size in the presence of cysts that are secreting low levels of

progesterone. Possibly, cysts can contribute to their replacement with new cysts through this mechanism (Silvia *et al*. 2002). In fact, cystic follicle at the advanced stage produces enough amount of progesterone (Calder *et al*. 2001; Isobe *et al*. 2005; Table 1)

DELAY OF FOLLICULAR REGRESSION

So far, disorder of ovulation has been focused mainly for the etiology of follicular cyst in dairy cows. However, if large anovulatory follicle is regressed immediately after the time when follicle reaches the diameter equivalent to that of follicle that ovulates, its follicle does not grow further more, resulting in no cystic follicle formed. Therefore, we propose that another cause of cystic follicle is a delay (lack) of degeneration system of follicle.

Atresia is the process of physiological degradation which affects all follicular components and progressively leads to elimination of the follicle. In cows, the granulosa cells in the atretic follicles are reported to be deleted by apoptosis (Jolly *et al*. 1994; Isobe and Yoshimura 2000a; Feranil *et al*. 2005). Therefore, it is strongly suggested that apoptosis plays a crucial role in the regression process of atretic follicles. Since excess growth of cystic follicle may be due to the lack or decline of degradative control in the follicular tissues, it is hypothesized that the unbalance of cell proliferation and apoptosis in the follicular cells may be responsible for this mechanism. Therefore, we investigated the localization of proliferating cells and apoptotic cells in the granulosa and theca layers of cystic follicles. In both atretic and cystic follicles, apoptotic cells are present in the granulosa and theca layers, although the changes in frequencies of apoptotic cells in the theca interna during progressive process was different between

atretic and cystic follicles (Isobe and Yoshimura 2000a). Cell proliferative activity in the granulosa and theca layers of cystic follicle are weak compared with those of in atretic follicle (Fig 2, Isobe and Yoshimura 2000b). These results suggest that the balance between cell proliferation and cell death is much different between cystic and atretic follicle. Since atretic follicle is normal follicular degenerative process, we assume that disorders in the balance between cell proliferation and apoptosis may be associated with the formation of cystic follicle (Isobe and Yoshimura 2000b).

Reduction of the capillary network leading to the deficiency of blood supply may be an important event for the regression of atretic follicles (Hay *et al*. 1976; O'Shea *et al*. 1978; Macchiarelli *et al*. 1993). Compared with the nonatretic healthy follicles in which capillaries are uniformly distributed, the microvasculature network became reduced in atretic follicles (Hay *et al*. 1976; O'Shea *et al*. 1978; Macchiarelli *et al*. 1993). A significantly greater induction of von Willebrand factor (vWF) was observed in the atretic follicles compared to the healthy follicles (Isobe et al. 2001). The production of vWF indicates damage of endothelial cells in the regressing blood vessel (Augustin *et al*. 1995; Bowyer *et al*. 1989; Karadogan *et al*. 2000; Murakami *et al*. 1988). Unlike atretic follicles, since cystic follicles are kept unregressed with ovulatory failure, disorder of vWF induction is possible in the cystic follicles. Therefore, we investigated the localization of vWF in the cystic follicle and compare that with atretic follicle. In the theca layers, vWF production decreased in the cystic follicles compared to atretic follicles (Fig. 3, Isobe *et al*. 2002). These results suggest that the reduction of vWF in the cystic follicles suppresses the degeneration of vascular system. Continuation of stability in vasculature may be one of the factors that delays the tissue regression in the cystic follicles, and also contributes to the accumulation of follicular

fluid that originates from the serum. Furthermore, in the experiment using *Bandeiraea simplicifolia*-I (BS-I) lectin to visualize the endothelial cells of whole microvessels, theca interna of cystic follicles had significantly greater microvessel distribution than healthy follicles. The mRNA and protein of vascular endothelial growth factor (VEGF), a potent mitogen for endothelial cells and a stimulator of vascular permeability, were revealed in the cystic follicles (Isobe *et al*. 2005). These results demonstrate that cystic follicles have a highly developed vasculature network and VEGF production.

CONCLUSION

The deficient feedback control between gonad and hypothalamus is likely primary cause of disorder of ovulation. More precise underling endocrine system will be further investigated using the animal model suffered cystic follicle artificially. Mechanism of growth of cystic follicle due to the delay of regression system remains to be elucidated. These two different approaches for cystic follicle formation will provide us valuable information that is helpful for the appropriate clinical treatment in dairy cows.

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Figure legend

Figure 1 Light microgpraphs of HE stained cystic follicles. (a) Follicular cyst with granulosa layers. (b) Follicular cyst without granulosa layer. (c) Luteal cyst. G: Granulosa layers, TI: Theca interna, LC: luteal cells

Figure 2 Light microgpraphs of healthy (a), atretic (b) and cystic (c) follicles immunostained with proliferating cell nuclear antigen (PCNA) antibody. Arrowheads represent PCNA-positive cells. G: Granulosa layer, TI: Theca interna, TE: Theca externa.

Figure 3 Light micrographs of the bovine atretic (a) and cystic (b) follicles immunostained for anti-von Willebrand factor (vWF). Arrowheads show examples of vWF-positive blood vessels. Arrows show examples of vWF-negative blood vessel. G: Granulosa layer, TI: Theca interna, TE: Theca externa. Bars = 50 μm.

Table 1 Concentrations of estradiol-17β, testosterone and progesterone in follicular fluid of cystic follicles with (GC+) and without (GC-) granulosa layer and healthy follicles

Follicle types	n	Concentration (ng/mL) of			Estradiol-17β/ Progesterone
		Estradiol-17β	Testosterone	Progesterone	
Cystic (GC+)	12	319.0[a] (48.9-720.6)[c]	16.2[a] (0.0-61.6)	28.2[a] (9.8-50.4)	12.2[a] (2.7-23.6)
Cystic (GC-)	6	15.4[a] (7.2-22.8)	1.4[a] (0.9-1.8)	316.5[b] (182.0-544.4)	0.06[b] (0.02-0.11)
Healthy (> 9 mm)	7	388.6[a] (75.5-1551.6)	27.8[a] (3.6-95.8)	64.0[a] (8.9-226.8)	9.1[a] (1.1-21.3)

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[a,b] Values within column with no superscripts in common are different at least $P < 0.05$.

[c] Range of value between minimum and maximum.

乳牛における卵胞嚢腫

磯部直樹

広島大学大学院生物圏科学研究科

広島県東広島市鏡山 1-4-4

卵胞嚢腫は乳牛において最も重要な繁殖障害であり、これに罹患すると卵巣機能に悪影響を及ぼし、空胎期間が延長する。したがって、この病気は酪農業界に莫大な損失をもたらす。本総説では卵胞嚢腫の 2 つの要因（排卵の障害および退行の遅延）に分けて議論する。排卵の障害は従来から卵胞嚢腫の原因として知られており、これはエストロジェンの視床下部へのフィードバック機能が障害を起こすことにより LH サージが欠如することによって引き起こされる。一方、もし排卵に障害を起こした後、卵胞の発達を続けることなく速やかに退行すれば卵胞嚢腫に陥ることはないと考えられる。したがって、もう一つの嚢腫卵胞の原因として卵胞退行の遅延（欠如）を提案する。本総説では、乳牛の卵胞嚢腫に関するこれら二つの原因それぞれについて最近の知見を紹介する。

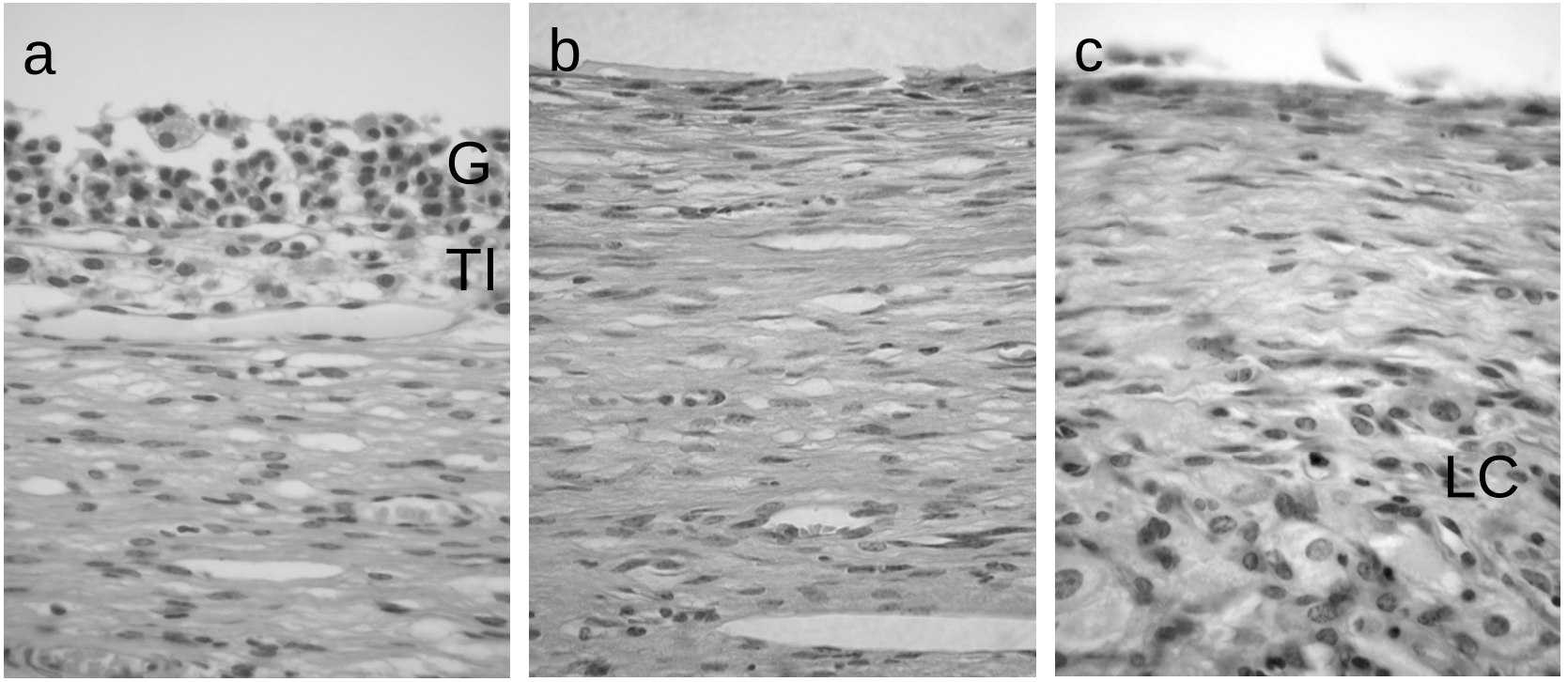

Fig. 1

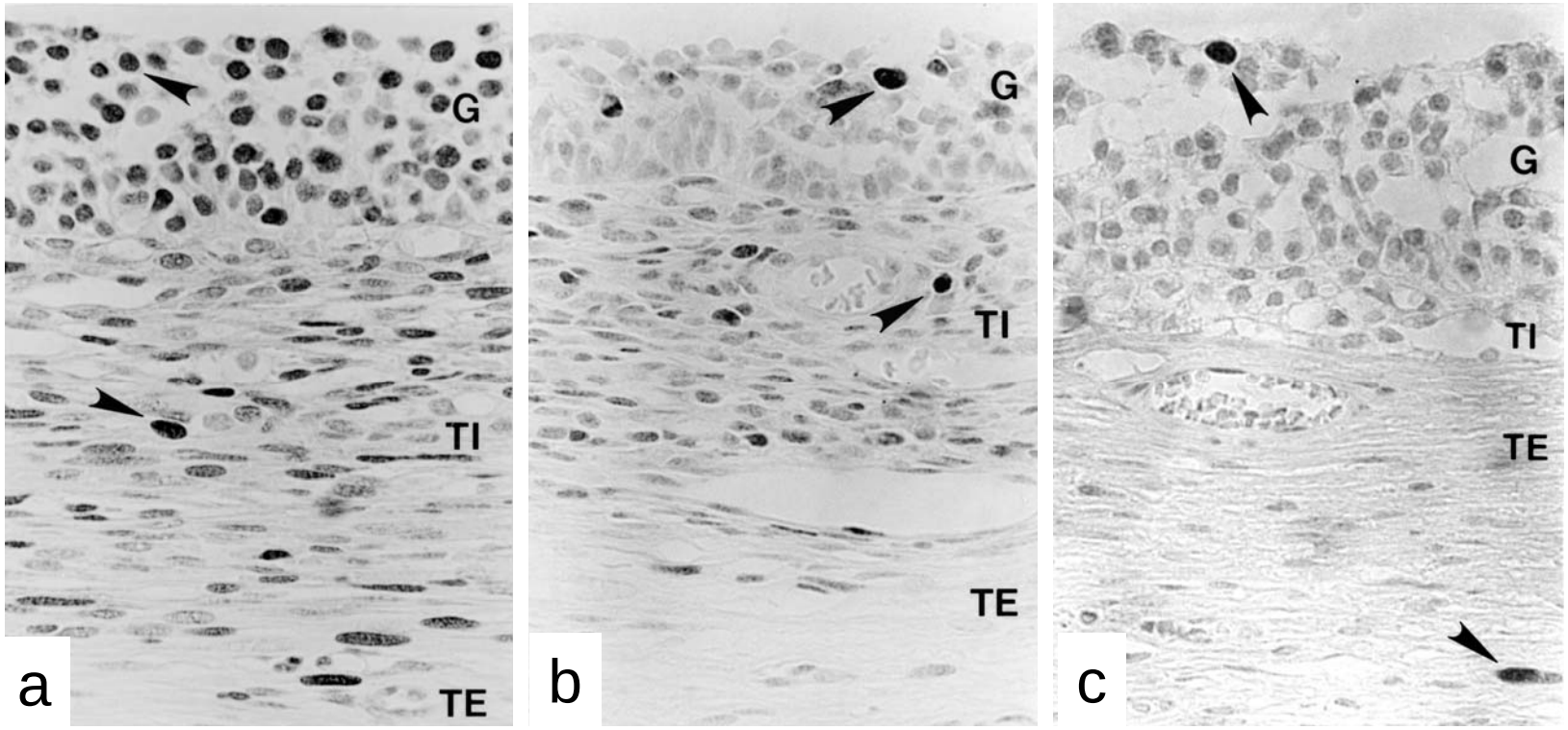

Fig. 2

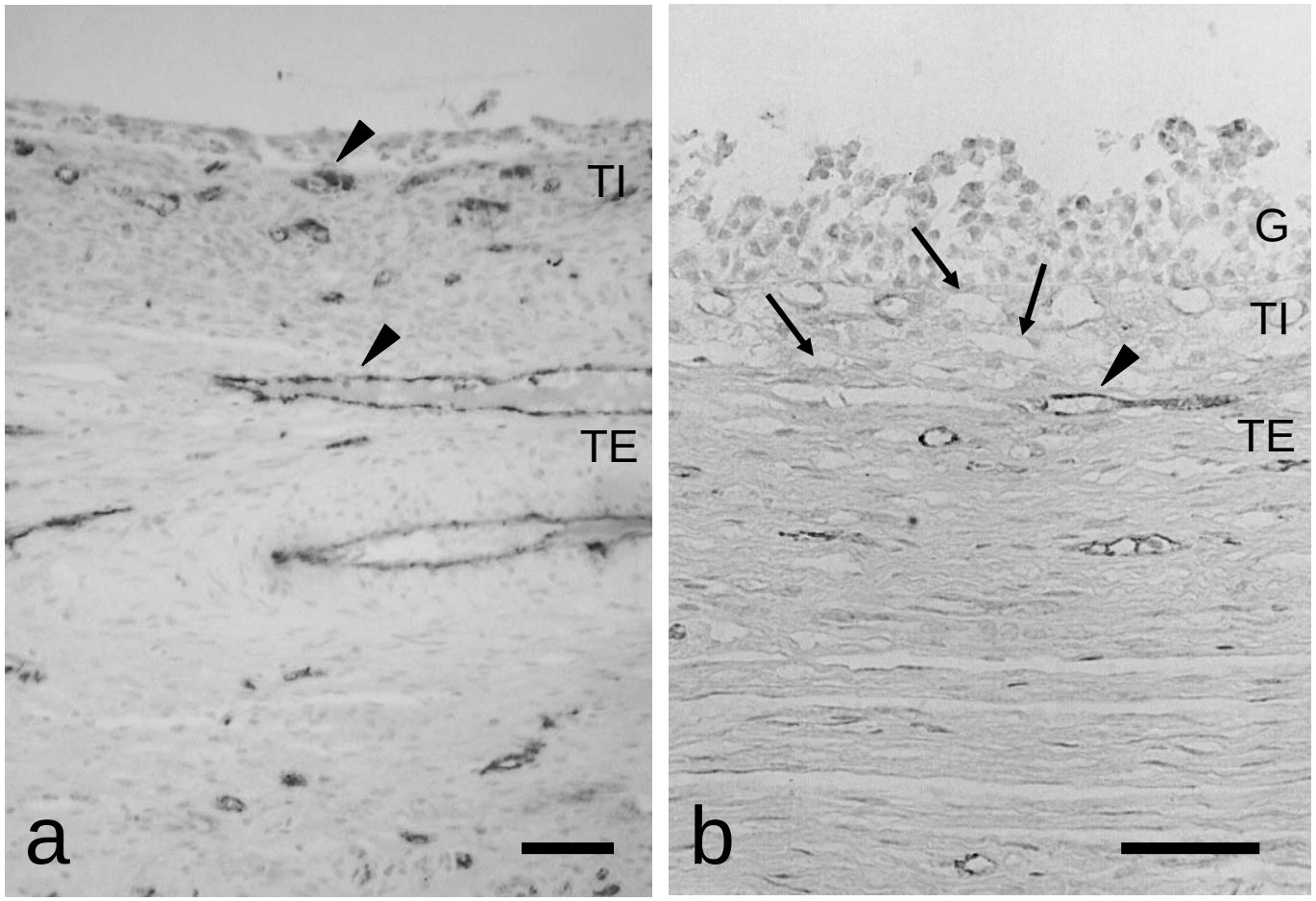

Fig. 3