OBSERVATIONS BY SCANNING ELECTRON MICROSCOPY OF OVIDUCTAL EPITHELIAL CELLS FROM BACTRIAN CAMEL (*Camel bactrianus*) AT FOLLICULAR AND POSTOVULATORY PHASES

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ABSTRACT

The luminal surface of epithelial cells in various regions of oviducts of the bactrian camel at the follicular phase and postovulatory phases of the oestrous cycle were examined by scanning electron microscopy. Cyclic changes appeared to most significant in the fimbriae, less apparent in the ampulla and ampullar-isthmic junction, and hardly visible in the isthmus. Significant changes were not observed in the height of cilia between follicular and postovulatory phase. Some cilia still existed in the epithelium at the postovulatory phase, the apices surfaces of the nonciliated cells were extremely variable, and had a few microvilli.

Key words: Bactrian camel, cyclic changes, deciliation, epithelial cells, scanning electron microscopy

The oviduct in mammals is an important organ where events about reproduction happen, such as maturation of gametes, fertilisation and the early development of embryos. Two types of cell, the ciliated cells and the nonciliated or secretory cells, are clearly discernable in the epithelium of the mammalian oviduct. Ciliated cells play an important role in the transport of oocytes into the oviduct, while nonciliated cells are very important in the reproductive and developmental events that occur in the oviduct. A considerable amount of information is available on the details of cyclic variation of the epithelial cells of the oviducts in a variety of the spontaneous ovulators, such as Chinese Meishan pig (Abe and Oikawa, 1992), cow (Abe and Oikawa, 1993) and goat (Abe et al, 1993).

The camel is a seasonally induced ovulator. Apart from 2 studies about the oviduct of dromedary camel (Nayak, 1977; Singh *et al*, 2005), there have been no reports on the extent to which cyclic changes occur among the oviducts of bactrian camel. The scope of the present study is to determine the cyclic variation of epithelium cells of the oviducts of bactrian camel.

Materials and Methods

The present study was conducted on the oviducts of 10 adult bactrian she-camels slaughtered at a local abattoir in inner Mongolia province of China.

As the behaviour of the bactrian camel in oestrous season was not as obvious as other livestock, the particular phase of oestrous cycle was determined from the physiologic state of the ovaries and records of the reproductive history of the animal (Nayak, 1977). Five camels were examined at 2 stages during the oestrous cycle: the follicular phase and the postovulatory phase. The oviducts were removed and placed in phosphate-buffered saline (PBS; pH=7.4) and subsequently trimmed. The fimbriae, ampulla, ampullar-isthmic junction and isthmus were cut apart and processed for scanning electron microscopy. The first fixation was carried out in 3% glutaric dialdehyde in 0.1M phosphate buffer. After the fixation, the specimens were washed in the PBS for 3 times, each for 10 minutes. The tissues were fixed again for one hour with the osmium tetraoxide and dehydrated in ascending grade ethanol solutions. The samples were then critical point dried and subsequently sputter coated with 35 nm thick layer of gold. The specimens were examined with a JEM-T330 scanning electron microscope operated at 20kv or 30kv and desired photographs were taken.

Results

Fimbriae

The SEM studies on oviduct epithelium of bactrian camels showed that ciliated cells appeared

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to predominate throughout the region of the fimbriae at the follicular phase. The cilia were fairly uniform, usually extending above the apices of nonciliated cells. Some droplets, presumably of secretory materials, were on the tips of some cilia. Numerous short microvilli protruded from the apical surfaces of nonciliated cells (Fig 1). Under a higher magnification, most of the cilia had a similar orientation. The microvilli were unevenly distributed on the apices of nonciliated cells, which appeared to be either flat or gently rounded in shape (Fig 2).

At the postovulatory phase, many bulbous processes of nonciliated cells bulged above the cilia. Most of the processes of nonciliated cells were elliptical in shape, lacked microvilli but not uniform in arrangement. Some black holes appeared in the epithelium of fimbriae (Fig 3). The cilia appeared to be partly hidden below the bulbous processes of the nonciliated cells. But it was possible to find some places in which microvilli were as dense as those of at the follicular phase. Marked changes were not observed in the length of the cilia (Fig 4).

Ampulla

The epithelium in the ampullar region of the follicular phase showed extensive ciliation as that of the fimbriae did at the follicular phase (Fig 5). The cilia extended above the apical processes of nonciliated cells. The nonciliated cells might bulge with an irregular bud, which had some microvilli on its base equal in height with the surrounded cilia. The bases of plicas were mostly non-ciliated cells; while on the tips of plicas were mostly ciliated (Fig 6).

At the postovulatory phase, the cilia were partly concealed by the processes of nonciliated cells, and were not as dense as at the follicular phase. The apices surfaces of the nonciliated cells were extremely variable, and had a few microvilli (Fig 7). The length of the cilia had also few changes.

Ampullar-isthmic junction

The epithelium in the Ampullar-isthmic junction of the follicular phase showed similar ciliation to that of the ampullar epithelium at the follicular phase. The apices surfaces of the nonciliated cells were concealed by the cilia (Fig 8).

At the postovulatory phase, the nonciliated cells were prominent on the epithelium, but the length of cilia had also no apparent changes. The apices of processes had a variety of sizes (Fig 9). The cilia were rarer than at follicular phase. The bulbous processes of nonciliated cells had few microvilli (Fig 10). The caudal of the oviduct showed little change in ciliation between the follicular and postovulatory phases. At the follicular phase, mostly, the protrusion of nonciliated cells were not conspicuous. Some black holes resulted from cells fallen from the epithelium were observed (Fig 11). Under a higher magnification, the apical surfaces of nonciliated cells were round, bulged above the epithelium, apparently had many microvilli. Some bulbous processes were emerged from the surface of nonciliated cell (Fig 12).

At the postovulatory phase, the apical surfaces of nonciliated cells were mainly in 3 states. First, they were round just as at the follicular phase. Second, the apical surfaces of some nonciliated cells were depressed to be discoidal or bowl-shaped. Third, they were extremely variable because of the cellular fragments on the apical surfaces (Fig 13). The height of the process was similar to the length of surrounding cilia. The nonciliated cells, in cobblestone arrangements, were partly concealed by the cilia. The apices surfaces of the processes of nonciliated cells lacked microvilli, but between them there were a few (Fig 14). In addition to mature cilia, there were a number of ciliated cells with short and stubby cilia (Fig 15).

Discussion

The present study revealed that the oviducts of the bactrian camel underwent an impressive change in ciliation during the oestrous cycle. Cyclic changes appeared to be most significant in the fimbriae, less apparent in the ampulla and ampullar-isthmic junction and were hardly visible in the isthmus. The fimbriae and ampullar regions showed extensive ciliation, but the quantity of cilia decreased greatly at the postovulatory phase, similar to that of Chinese Meishan pig (Abe and Oikawa, 1992), cow (Abe and Oikawa, 1993) and goat (Abe et al, 1993) at the postovulatory phase. In the fimbriae and ampullar regions, the important function of cilia was considered to be primarily responsible for the pickup and transport of ovulated eggs. Therefore, the change of cilia between the follicular phase and the postovulatory phase might demonstrate the function of the cilia.

Unlike above-mentioned spontaneous ovulators, significant changes were not observed in the height of cilia between the follicular phase and the postovulatory phase. Similar findings were seen in the infundibular region of one-humped camel (Nayak, 1977). The bactrian camel is a seasonally induced-ovulator, with the follicular waves instead

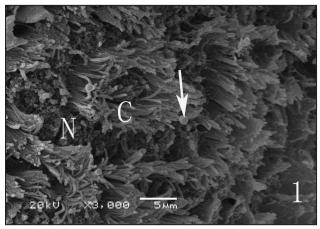


Fig1. Fimbriae from the oviduct of a bactrian camel in the follicular phase showing extensive ciliation. The cilia (C) were fairly uniform, usually extending above the apices of nonciliated cells (N). A droplet (arrow), presumably of secretory origin, appeared on the tip of cilia.

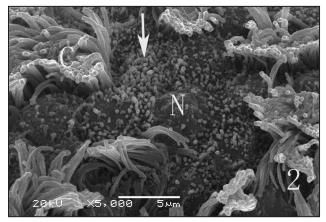


Fig 2. Fimbriae from the oviduct of a bactrian camel in the follicular phase. The microvilli (arrow) were unevenly distributed on the apices of nonciliated cells (N), which appeared to be either flat or gently rounded in shape.

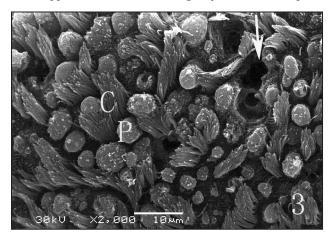


Fig 3. Fimbriae from the oviduct of a bactrian camel in the postovulatory phase showing the conspicuous processes of nonciliated cells (P). Most of the processes of nonciliated cells lacked microvilli. Some black holes appeared in the epithelium (white arrow).

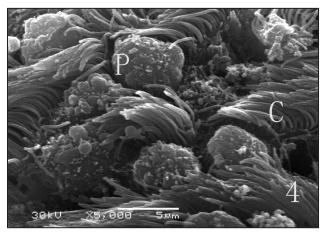


Fig 4. Fimbriae from the oviduct of a bactrian camel in the postovulatory phase. The cilia (C) appeared to be partly hidden below the bulbous processes (P) of the nonciliated cells.

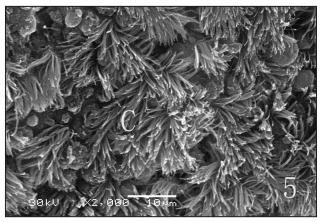


Fig 5. Ampulla from the oviduct of a bactrian camel in the follicular phase. The epithelium is extensively ciliated. The cilia (C) extend above the apical processes of nonciliated cells.

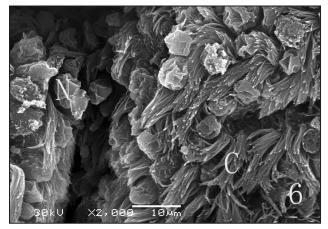


Fig 6. Ampulla from the oviduct of a bactrian camel in the follicular phase. The bases of plicas were mostly nonciliated cells (N); while on the tips of plicas were mostly ciliated (C).

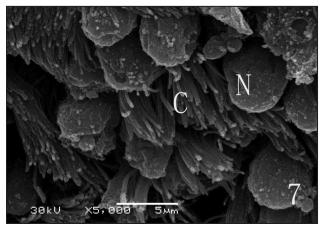


Fig 7. Ampulla from the oviduct of a bactrian camel in the postovulatory phase. The cilia were partly concealed by the processes of nonciliated cells (N). The apices surfaces of the nonciliated cells were extremely variable, and had few microvilli.

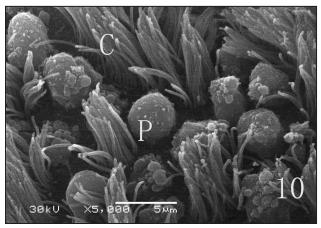


Fig 10. The ampullar-isthmic junction from the oviduct of a bactrian camel in the postovulatory phase. The cilia were rare and the surfaces of bulbous processes (P) of nonciliated cells had few microvilli.

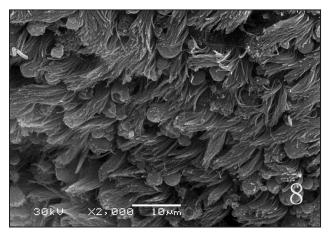


Fig 8. The ampullar-isthmic junction from the oviduct of a bactrian camel in the follicular phase showed similar ciliation to the fimbrial and ampullar epithelium.

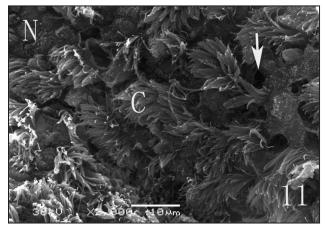


Fig 11. Isthmus from the oviduct of a bactrian camel in the follicular phase. The epithelium is not extensively ciliated. There were black holes (arrow) resulted from cells fallen from the epithelium.

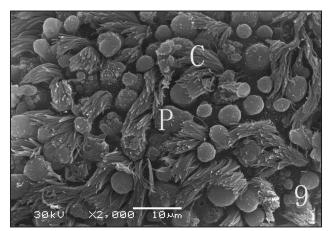


Fig 9. The ampullar-isthmic junction from the oviduct of a bactrian camel in the postovulatory phase. The cilia (C) were partly concealed by the bulbous process (P) of nonciliated cells.

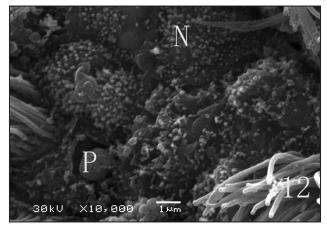


Fig 12. Isthmus from the oviduct of a bactrian camel in the follicular phase. The apical surfaces of nonciliated cells (N) were gently rounded, and had many microvilli. A bulbous process (P) was emerged from the surface of epithelium.

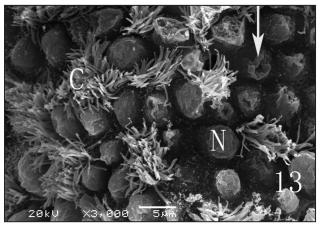


Fig 13. Isthmus from the oviduct of a bactrian camel in the postovulatory phase. The apical surfaces of some nonciliated cells were depressed to be discoidal or bowl-shaped (arrow).

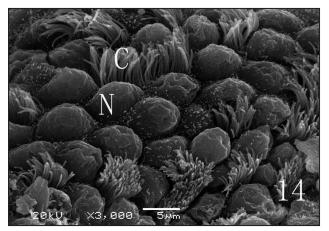


Fig 14. Isthmus from the oviduct of a bactrian camel in the postovulatory phase. The nonciliated cells (N), in cobblestone arrangements, were partly concealed by the cilia (C).

of the oestrous cycle of the spontaneous ovulators during the breeding season (Zhao *et al*, 1998). They would discharge ovum only after mating or injecting seminal plasma. If the ovum was not fertilised, they would discharge another ovum after mating during the oestrous season. An exclusive feature of bactrian camel in the follicle cycle is that they would not reject to mate until 2 to 8 day after ovulation. Therefore, it is possible to ovulate consecutively in the oestrous cycle. Proper cilia in the fimbriae and ampullary regions may be good for pickup and transportation of the next egg at the postovulatory phase.

It is well established that the regular cycle of ciliogenesis and deciliation in mammalian oviducts is dependent upon the levels of circulating oestrogen and progesterone. According to Zhao *et al* (1998), the content of oestradiol-17 in the peripheral

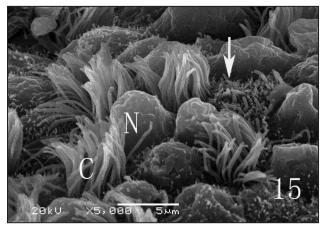


Fig 15. Isthmus from the oviduct of a bactrian camel in the postovulatory phase. In addition to mature cilia (C), there were a number of cells with short and stubby cilia (arrow).

blood after ovulation decreased obviously, while the progesterone was increased significantly, although during the cycle they had some changes. The deciliation may be argued to the change of the hormones level in the blood. On the contrary, the study of De Hollis *et al* (1984) and Rong (2003) demonstrated that there were almost no deciliation in the oviducts of Merino ewes and rat. That might be because their genital cycle were very short, the synthesis and secretion of steroid hormone were too limited to make the shedding of cilia at the postovulatory phase.

The actual mechanism that caused deciliation appeared to remain at least partially unknown (Odor *et al*, 1980). In the epithelium of oviducts in bactrian camel, some black holes were observed in the isthmus region. They may result from the shedding of ciliated cells, which was one of the 3 mechanism had been summarised by Odor *et al* (1980). In this study, some short and stubby cilia were shown in the epithelium of isthmus of oviducts at the postovulatory phase. These cells appeared to be in different phases of ciliogenesis.

Marked changes in nonciliated cells were observed in all investigating segments. At the postovulatory phase, the protrution of nonciliated cells were obvious and an interesting phenomenon was the apical surfaces of some nonciliated cells depressed and had discoidal or bowl-shape. Similar cyclic changes have been observed in human (Liu and Kuang, 1989). The nonciliated cells in different shapes might be in process of secretion. In different phases of secretion, the contents of cytoplasm in nonciliated cells might differ. Hence, the apical surfaces of nonciliated cells had a variety of shapes. The cytoplasmic bud on some apices of nonciliated cells might be secretory materials gathered on the epithelium and when the secretory activities weakened, the cytoplasm synthesised by regulation to make them protrude again. This variability of the tissue was also reported in previous study by Rumery *et al* (1978).

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