Ovarian Follicle Activity in Yak versus Cattle and Buffalo  
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Summary  
A comparison of the ovarian follicular system in cattle, buffalo and yak yields potentially useful information in developing methods for the improvement of reproduction efficiency in yaks. The ovaries in yaks were generally smaller than in both cattle and buffalo. The histological structures of the yak ovaries and follicular system resembled in all aspects those described for cattle and buffaloes. In yak, the average number of primordial follicles was markedly lower than in cattle, but higher than in buffalo. The number of growing follicles was higher than in cattle and buffalo. The number of Graafian follicles was lower than in cattle, but generally similar to that in buffalo. In conclusion, the number of follicles in each developmental phase in yaks is lower than in cattle, and higher than in buffalo. The possible roles of genetic and environmental factors in determining follicle status is discussed.

Introduction  
Yaks play a prominent role in the mountainous livestock production, particularly in the highland of Asia, and factors affecting productivity are of paramount importance to agricultural economics in this region of the world. Both yak and buffalos are adaptable to the extreme ecological conditions. Due to constriction of nutritional and ecological condition, their reproduction performance is known to suffer from a number of inherent problems that include late maturity, poor estrus expression, distinct seasonal reproductive patterns, prolonged intercalving intervals and low conception rates. Their fertility performance and reproductive efficiency show a distinct influence of time of year. The review made a comprehensive comparison of form and functions of ovaries among cattle, buffaloes and yaks with view to applying reproductive technology in cattle to yak and buffaloes and promoting the development of yak and buffalo production. All means are reported as mean +/- standard deviation.

Ovarian Anatomy  
In cattle the growth of ovaries is the most rapid from birth to puberty. The weights of both ovaries are almost the same at birth. At sexual maturity the right ovary is heavier. Settergren [1] demonstrated that the left ovary was generally heavier, but the difference between the right and left ovary was not significant. The length, width and thickness of the ovaries were almost the same for the right (25.1, 18.4 and 15.3 mm) and the left ovary (25.7, 19.1 and 16.1 mm) [1]. In buffaloes the ovaries are smaller than in cattle. However, there is considerable change in ovarian weight and dimensions during the different phases of the sexual cycles. Maximum size and weight were observed when a fully developed corpus luteum existed in the ovary The minimum and maximum average weights being 2.9 g and 6.1 g in buffaloes and 3.9 g and 9.9 g in cattle [2,3]. Danell reported that the average weight of the left and right ovaries in 30 normally cycling buffaloes were 3.4±1.3 g and 3.6±1.5 g, whereas in non-cycling animals the weights were 2.5±1.2 g and 2.5±0.9 g, respectively [4]. In yaks, ovarian weight and size increased with age. The average ovarian weight increased from 0.32 g in 1-month-old calves to 2.12 g in 7-years-old yak cows. The average length, width and thickness of ovaries increased from 1.18, 0.70 and 0.44 cm to 2.15, 1.66, 1.10 cm. No significant differences were found between the weight and size of yak ovaries within the same age group [5]. The results indicate that the weight and size of yak ovaries are less than those of cattle, but similar to those of buffalos.

Ovarian Histology in Yaks  
The histological structure of yak ovaries resembled in all aspects the description of cattle ovaries [1,6-8] and buffaloes [3,4-9]. The covering epithelium, is about 5 - 10 µm and usually consists of simple columnar and cubical, or sometimes squamous epithelial cells, although stratified epithelial cells are sometimes observed in neonates. The nuclei of the epithelial cells are round, oval or flat and often rich in chromatin. The covering epithelium of ovaries is invaginated into the cortical stroma to form tubule-like structures lined with columnar or sometimes cubical cells in the neonates and heifers, and sometimes in
The ovaries have a similar number of primordial follicles [4]. The population of primordial follicles is estimated to be about 19 million. The numbers of primordial follicles in the ovary is lower in buffaloes and yaks than in cattle. Left (49.3%) and right (50.7%) ovaries have a similar number of primordial follicles [4]. The population of primordial follicles is estimated to be about 19 million.

**Follicular Development**

The atresia of Graafian follicles can be divided into the early, definitive and late stages. Early atresia appears in synchrony with a prolonged period of follicular activity. In cattle and buffalo, the cortex contains the primordial follicles, which usually contain one oocyte but sometimes two, rarely three. The growing follicles with two or three layers of follicular epithelium are situated deeper into the cortex than primordial follicles. The connective tissue around the growing follicles forms the theca that later differentiates into theca interna and theca externa.

Primordial follicles in yak are distributed in the peripheral part of the cortex, frequently rather close to the tunica albuginea. Primordial follicles usually contain one, but occasionally two oocytes surrounded by a single layer of squamous epithelial cells. In newborn calves, primordial follicles are generally distributed in groups, usually more than 10 grouped together [10].

The growing follicles are usually deeper into the cortex of the ovary with two or more layers of polygonal or cubical epithelial cells. The size of growing follicles is 80 - 120 µm in diameter and their oocytes are 40 - 45 µm, with nuclei of about 15 µm. When the oocyte is surrounded by 4 - 5 layers of epithelial cells, the surrounding connective tissue starts to form what will become the theca layers and small pools of fluid starts to form among the epithelial cells.

The young Graafian follicles, characterized by the antrum formation, are surrounded by three or four layers of granulosa cells resting on a basement membrane. In general, when follicles enlarge to 300 - 500 µm, one complete antrum forms. At this time, the oocytes are 60 - 90 µm with nuclei of 35 - 40 µm. The granulosa cells next to the basement membrane are larger than other cells, with regularly arranged nuclei. The theca layer consists of two parts: the theca externa and theca interna, as in cattle and buffalo. Blood vessels of the theca externa supply capillaries to the theca interna. The theca externa has indistinct boundaries [10].

**Atresia of Follicles**

Obliterative atresia with primary follicular wall degeneration in Graafian follicles is described in cattle [11] and divided into three degrees. The same type of atresia is by far the most common in buffalo heifers. Danell [4] described two degrees of atresia in buffalos. The first degree of atresia was characterized by a number of pyknotic nuclei in the liquor folliculi and in the granulosa layer of the follicular wall. The beginning of the second degree of atresia is characterized by changes in the granulosa layer, alone, with few or no pyknotic nuclei in the antral fluid. Later, theca connective tissue cells are observed in the antrum. In the theca interna the epithelial cells disappear and the connective tissue cells predominate. Pyknotic nuclei are observed in the cumulus. Later, the cumulus disappears and only the naked oocyte remains and an ingrowth of connective tissue into the lumen takes place. The follicle finally becomes a corpus atreticum.

In a study on follicular population in cycling and non-cycling buffalo heifers, Danell observed that average number of follicles over 2.00 mm in diameter to be 16.8 and 23.6, respectively, and did not significantly differ. However, there were twice as many atretic follicles as normal ones (31.7 vs. 14.6, respectively) in cycling animals. The average atresia frequency for all animals was 76.6% [4]. A similar value of 82%, was obtained following histological examination of follicular atresia at random stages of reproduction in ovaries from swamp buffaloes obtained at slaughter [12].

The atresia of follicles in the yak can occur at any stage of follicular development, including Graafian follicles late in development. The atresia of Graafian follicles can be divided into the early, definitive and late stages. Early atresia appears in three forms: loosening and sloughing of granulosa cells lining the antrum; disappearance of the membrana propria and loss of orientation of the basal layer of the granulosum and shortening and rounding of the theca interna cells. The definitive stage of atresia in yaks, which is also described in dairy cows, is characterized by collapsing, contraction or cystic appearance. Collapsing atresia constitutes about 15% of the total follicular atresia. Contracting atresia is the most common type of follicular regression, it is observed as the only type of atresia in many follicles, and comprises more than 80% of all the definitive atresia. Cystic follicular atresia occurs infrequently, comprising less than 5% of all the definite atresia. Late-stage atresia is the final stage of regression common to all types of atretic vesicular follicles. The size is reduced and cell layers are disorganized, the antrum becomes gradually filled with fibrous granulosa remnants and the theca layers become hyalinized [10].

**Follicular Development**

The numbers of primordial follicles in the ovary is lower in buffaloes and yaks than in cattle. Left (49.3%) and right (50.7%) ovaries have a similar number of primordial follicles [4]. The population of primordial follicles is estimated to be about 19 million.
000 in Nili-Ravi buffaloes [13] and 12 000 in Sarti Buffalo [4], 32 870 in 2-year-old yaks [10], compared to between 60 000 and 100 000 in the cow. This suggests a reason for the lower reproductive potential of buffaloes and yaks. In the cow, the number of ovulations following gonadotrophin treatment is related to the number of healthy follicles over 1.7 mm in diameter present before initiation of treatment [14]. Since the number of such follicles has been found to range from one to five in buffaloes, in comparison with a range of 17 - 32 in cows, the low follicular population may contribute to the low superovulatory response in buffaloes [15].

According to Cui [10], the number of primordial follicles in yaks is much lower than in cattle, but higher than in buffaloes. The average total number of primordial follicles per ovary pair in 1-month-old calves, 1-year and 2-year-old heifers and 7 to 10 year old yak cows were 53.5±6.3, 32.9±4.5, 22.9±2.8 and 9.5±1.2 respectively. On average, there were about the same number of primordial follicles in the left and the right ovaries among each age group. However, a great difference existed between the two ovaries in an individual animal. Although the number of primordial follicles appears to be considerably lower in buffaloes and yaks than in cattle, the effect of age on population has apparently not been evaluated in buffaloes and yaks. In addition, buffalo ovaries contain only about 20% of of the antral follicles found in cattle ovaries [15].

Erickson [16] reported that the number of growing follicles per ovary pair in 22 to 50 day-old Herefords, 12 months old, 19 to 24 months old and 7 to 9 years old were 93±18, 248±33, 233±38 and 154±15 respectively. The study in yaks showed that the average total number of growing follicles per ovary pair in 1 month-old calves, 1 year-old heifers, 2 year-old heifers and to 10 year old yak cows were 210±76, 815±95, 895±142 and 445±88, respectively [10]. There are significant differences between age groups.

Rajakoski [8] carried out quantitative estimation of the number of Graafian follicles ≥1 mm in cattle with reference to seasonal, cyclical and left-right variations. Normal and atretic follicles ≥1 mm were found in equal numbers, on an average 46.3 and 46.0 respectively in each pair of ovaries. He found that cyclical differences in the number of follicles ≥5 mm indicated two growth waves during a sexual cycle. One hundred and thirty ovaries contained a similar number of follicles ≥1 mm, however, there was a greater number of normal follicles ≥5 mm in the right than in the left ovary. Several subsequent studies using similar techniques further supported the theory of follicular waves [1,17,18], although these studies relied on single time-point data. Using ink marking at laparotomy, it was demonstrated that ovulatory follicles only became the largest follicle on the ovary within 2 to 3 days of oestrus, and confirmed the dynamic nature of ovarian turn-over [19-21]. More recent studies using transrectal real-time ultrasound techniques have confirmed and extended the previous histological and gross morphological data showing two and three major phases of growth of large follicles during the bovine estrous cycle. The ovulatory follicle is selected around 3 days prior to ovulation [22-26].

In cattle each wave of follicular development is characterized by simultaneous emergence of medium-sized (>4 mm in diameter) growing follicles from a pool of smaller follicles. One of these follicles rapidly emerges as the dominant follicle (>7 mm in diameter) and continues to develop, while the others undergo atresia and regress. In cattle it usually takes 5 to 7 days for the dominant follicle to reach ovulatory size [27,28]. The dominant follicle normally reaches a maximum size of about 15 mm in diameter and remains dominant for a few days, until it becomes atresic and regresses, and is replaced within approximately 5 days by a new dominant follicle developing from the next wave of follicular development. If luteal regression occurs during the growth phase or early period of dominance, then the dominant follicle will continue to develop to preovulatory size (up to 20 mm in cattle) and will eventually trigger the hormonal cascade leading to ovulation [29].

In cattle follicular waves appear to be a constitutive characteristic since they are present prior to puberty [30], throughout most of pregnancy [31] and the post-partum period [32,33], as well as during estrous cycles. The number of waves per estrous cycle is usually two [34] or three [24,25] and reflects a genetic and environmental influence [35]. Singh et al. [36] delineated the pattern of development and atresia of large follicles (>8 mm) on the surface of ovaries of buffalo heifers. The authors concluded that their findings agreed with Rajakoski’s theory that the follicles at midcycle become atretic and that a new growth wave of follicles begins around midcycle and gives rise to the follicle(s) which would ovulate after estrus [8].

Danell [4] reported that the average number of Graafian follicles in the ovaries of cycling buffalo heifers was 46.3 and in the non-cycling animals 57.89. The average number of follicles in the left and right ovaries was 23.8 and 22.5 in cycling heifers and 35 and 32.8 in non-cycling ones, respectively. Danell also indicated that a significant correlation existed between the number of primordial follicles and the number of Graafian follicles ≥1 mm (p = 0.048). Evaluation of the number of follicles of 2 mm on the ovaries was carried out at three different time periods of the estrous cycle and revealed a greater number of follicles between Days 1 - 8 and 9 - 11 than during Days 12 - 21 [4].

Compared with cattle and buffalo, there is little available data on Graafian follicles in the yak. One study on yaks [10] showed that the average total number of Graafian follicles in 1 month-old calves, 1 year-old heifers, 2 year-old heifers and 7 year-old cow were 36.5±14.2, 41.7±12.3, 37.8±9.8 and 42.5±14.5, respectively. The difference was not significant among age groups or between the right and left ovaries within each age group. The numbers of atretic Graafian follicles in 1 month-old calves, 1 year-old heifers, 2 year-old heifers and 7 to 10 year-old yak cows were 22.1±55.6, 21.2±7.6, 21.5±4.7 and 25.3±6.7, respectively. No significant differences exist between age groups. The time of sampling was during the reproductive season, although the animals showed no signs of estrus. Therefore, comparatively, in the non-estrus phase of the
cycle, the numbers of Graafian follicles in yaks were lower than in cattle, but basically similar to those in buffaloes.

Conclusion
From current available data on the development of follicles in yaks, it is clear that the follicular number at each developmental phase in yaks is lower than in cattle, but it is similar to in buffaloes. Buffalo and yak are both seasonal breeders in which ovarian function is greatly suppressed during extreme climatic conditions [15]. Conditions are typically harsher for yaks. It is well known that nutritional deficiency is one of the important conditions that limits production. Possible effects of nutrition on ovarian follicle function merits consideration.

There is a strong need to identify the factors responsible for the low reproductive efficiency in yaks. Available information on the patterns of follicle development in yaks is inadequate and further study is needed. Further studies are needed to understand the processes of follicle recruitment, development and atresia and the temporal patterns of follicle selection, dominance, follicle numbers and preovulatory changes and follicular dynamics using techniques which permit serial assessment of changes occurring overtime. Emphasis may be directed towards investigating follicular waves as a functional unit. The knowledge obtained from such basic research may then be used to develop and test models for enhancing yak reproductive efficiency.

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