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Histochemical localization of mucopolysaccharides, proteins and lipids in ovarian tissue: A seasonal study

JASVINDER SINGH SASAN[1], VARINDER UPPAL[2] and NEELAM BANSAL[3]

Guru Angad Dev University of Veterinary and Animal Sciences, Ludhiana, Punjab 141004 India

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ABSTRACT

The present research work was conducted to study histochemical distribution of mucopolysaccharides, basic proteins and lipids in ovarian tissue during different seasons of year. For this purpose, 100 ovaries of adult buffaloes during different seasons (20 in each season) of year, viz. winter (November - January), spring (February – March), summer (April - June), rainy (July - August) and autumn (September - October) were collected from slaughterhouse. The paraffin sections of 5–6 µm were cut and stained with Periodic Acid Schiff, Alcian blue and bromphenol blue to localize neutral and acid mucopolysaccharides and basic proteins whereas for demonstration of lipids, cryostat sections of fresh ovarian tissue were cut and stained with Sudan black B.The distribution of different histochemical moieties in ovarian tissue did not vary among seasons but differs according to the physiological status of the ovary. A strong reaction of acid mucopolysaccharides in atretic follicles may be an indicator of follicular atresia. Increased amount of sudanophilic lipids was observed in primordial, primary follicles and luteal cells of corpus luteum.

Key words: Buffalo, Histochemistry, Lipids, Mucopolysaccharides, Ovary, Protein, Seasons

Buffalo reproduction is very susceptible to extreme conditions of temperature. The breeding frequency in buffaloes is highest during winters, decreased in autumn and spring season and lowest in summer (Shah 1988). Thus, the temperature has a very potent effect on the ovarian function and thus fertility in buffaloes. The mucopolysaccharides, proteins and lipids play an important role in follicular development so it is important to study their distribution pattern during different seasons of year. Therefore, the present study was conducted to establish the histochemical norms of buffalo ovary during different seasons. This will be contributory to the existing anatomical knowledge and that will form a basis for further investigations.

MATERIALS AND METHODS

The present study was conducted on left and right ovaries of 100 adult buffaloes during different seasons (20 in each season) of year, viz. winter (November - January), spring (February – March), summer (April - June), rainy (July - August) and autumn (September - October) collected from slaughterhouse. The ovaries were processed by acetone benzene schedule and paraffin sections of 5–6 µm were cut and stained with Periodic Acid Schiff, Alcian blue and bromphenol blue to localize neutral and acid mucopolysaccharides and basic proteins. For demonstration of lipids, cryostat sections of fresh ovarian tissue were cut and stained with Sudan black B (Luna 1968).

Present address: [1]Assistant Professor, ACVM, Jaipur (jssasan216@gmail.com). [2] Associate Professor, [3] Professor and Head.

RESULTS AND DISCUSSION

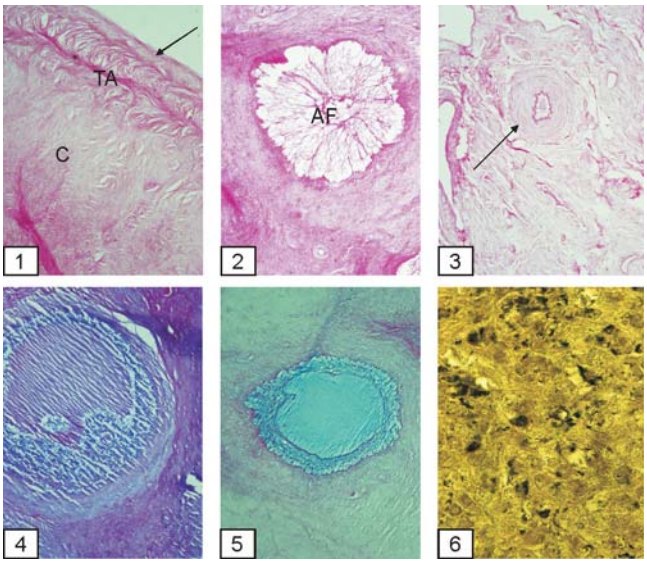
The histochemical distribution of neutral and acid mucopolysaccharides, basic proteins and sudanophilic lipids in different structures of buffalo ovaries during different seasons of year has been summarized in Table 1. The study revealed that the distribution of these histochemical moieties did not vary in different structures of ovary among seasons but the reaction was variable in ovarian tissue depending upon their physiological status.

Neutral mucopolysaccharides: The surface epithelium and tunica albugenia of adult buffalo ovary showed moderate reaction for neutral mucopolysaccharides (Fig. 1) whereas cortical and medullary stroma was weak to moderately positive as reported earlier by Bhardwaj (1996) in buffalo ovary. The primordial and primary follicles were also moderately positive. In growing and mature follicles, different parts of follicle showed different reactions. Theca cells were weakly positive in growing follicles and moderately positive in mature follicles whereas basement membrane, membrana granulosa and zona pellucida showed

Table 1. Histochemical distribution of mucopolysaccharides, basic proteins and lipids in the ovary of adult buffaloes

Stain	Histochemical moieties	SE	TA	S	Cortex															Medulla		
				Pd/	Secondary				Tertiary				Atretic				CL					
				Pr	TC	BM	MG	ZP	O	TC	BM	MG	ZP	O	TC	BM	MG	ZP	O	Ct	LC	MS	RO	BV
Periodic acid Schiff	NMPS	++	++	++	+	++/	++/	++/	++	++	++/	++/	+++	++	++	+/++	++	++	++	++/	++	+/++	+++	+++/
						+++	+++	+++			+++	+++								+++	+			++++
Alcian Blue	AMPS	+/++	++	++	+	+/++	++	++	+	++	++	++	++	+	++	++	+++/	+++	+	++	++	+/	++	+/+
																	++++					++	+	+
Bromphenol blue	Basic proteins	+/++	++	++	+/++	++	++/	++	+/++	++	++	++/	++/	++	+/++	+/++	+/++	+	+	++	+/++	++	++	++
							+++					+++	+++											
Sudan black B	Lipids	+	+/++	+/	++	+	++	0/+	++	++	+	++	0/+	++	++/	+	++	0/+	++	+/++	+++	+	+/++	++
				++	+++										+++									

SE, surface epithelium; TA, tunica albugenia; TC, theca cells; BM, basement membrane; S, stroma; Pd, primordial follicle; RO, rete ovarii; CL, corpus luteum; LC, luteal cells; MS, medullary stroma; ZP, zona pellucida; 0 – negative; +, weak; ++, moderate; +++, strong; MG, membrana granulosa; BV, blood vessels; NMPS, neutral mucopolysaccharides; ++++ - intense; Pr; primary follicle; Ct, connective tissue covering; AMPS, acid mucopolysaccharides.



Figs. 1–6. **1.** PAS reaction in surface epithelium (arrow), tunica albugenia (TA) and cortex (C). PAS stain ×10. **2.** PAS reaction in atretic follicle (AF). PAS stain ×10. **3.** PAS reaction in blood vessel (arrow). PAS stain ×10. **4.** Positive reaction for acid mucopolysaccharides in normal follicle. PAS-AB stain ×10. **5.** Positive reaction for acid mucopolysaccharides in atretic follicle. PAS-AB stain ×10. **6.** Photomicrograph of cryostat section of ovary showing sudanophilic lipids in luteal cells of corpus luteum. Sudan Black B stain ×40.

a moderate to strong reaction. Oocyte was moderately positive and antrum was weak to moderately positive. In atretic follicles, theca cells, membrana granulosa, zona pellucida, oocyte and antrum were moderately positive (Fig. 2) whereas basement membrane was having weak to moderate reaction whereas Bhardwaj (1996) reported a strong reaction in membrana granulosa and zona pellucida. Rete ovarii showed strong reaction for neutral mucopolysaccharides and tunica intima of blood vessels were strong to intensly positive (Fig. 3). In case of corpus luteum, connective tissue covering was moderate to strongly positive whereas luteal cells showed moderate reaction. Bhardwaj (1996) has confirmed that the neutral mucopolysaccharide observed in luteal cells was glycogen.

Acid mucopolysaccharides: The surface epithelium showed weak to moderate reaction for acid mucopolysaccharides whereas tunica albugenia and cortical stroma showed moderate reaction, which is in consonance with observations of Bhardwaj (1996). Medullary stroma showed weak to moderate reaction. Primordial and primary follicles were also moderately positive as observed by Bhardwaj (1996). In both growing and tertiary follicles, membrana granulosa, zona pellucida and antrum showed moderate reaction whereas oocyte showed weak reaction. Theca cells of growing follicles were weakly positive whereas that of tertiary follicles showed moderate reaction (Fig. 4). Basement membrane showed weak to moderate

reaction in growing follicles whereas in tertiary follicles, it was moderately positive. Theca cells and basement membrane of atretic follicles were moderately positive whereas zona pellucida and antrum were strongly positive (Fig. 5). Membrana granulosa of atretic follicles showed strong to intense reaction whereas it was low in oocyte. It has been reported that surface carbohydrates of granulosa cells contain hyaluronic acid, sulphated glycosaminoglycans and chondroitin sulphate. These carbohydrates may be involved in adhesion of granulosa cells, thus playing an important role in follicular growth, atresia and luteinization (Prasad *et al*.1979). Bellin and Ax (1984) reported high concentration of chondroitin sulphate in the atretic follicles in cattle. In present study, all the atretic follicles showed a strong reaction for acid mucopolysaccharides so this high level of acid mucopolysaccharides in the atretic follicle can be used as an indicator of atresia.

Rete ovarii showed strong reaction and connective tissue covering and luteal cells of corpus luteum were moderately positive.

Basic proteins: The surface epithelium showed weak to moderate reaction for basic proteins. Tunica albugenia and both cortical and medullary stroma were moderately positive. Similar findings were observed by Gupta *et al.* (2006) in goat and Bansal *et al.* (2008) in cat ovary. Theca cells were weak to moderately positive in growing follicle whereas membrana granulosa was moderate to strongly positive in both growing and tertiary follicles. In growing follicles, zona pellucida and antrum were also moderately positive and oocyte showed weak to moderate reaction. In tertiary follicle, zona pellucida was moderate to strongly positive, oocyte was moderately positive and antrum showed weak to moderate reaction. In atretic follicle, theca cells, basement membrane and membrana granulosa were weak to moderately positive whereas zona pellucida and oocyte showed weak reaction. In medulla, both rete ovarii and blood vessels showed moderate reaction. In corpus luteum, capsule showed moderate reaction whereas luteal cells were weak to moderately positive for basic proteins.

Lipids: The surface epithelium of ovary showed low lipid contents. Tunica albugenia and cortical stroma showed weak to moderate reaction whereas Goswami (1985) reported overall negligible reaction for lipids in the tunica albugenia whereas medullary stroma showed a weak reaction. Primordial and primary follicles were moderate to strongly positive for sudanophilic lipids. Earlier highly sudanophilic lipid droplets have been reported in buffalo primordial and primary follicles which disappeared during further growth of the follicles (Guraya 1997). It has been suggested that these lipids play an important role in developmental process of follicular growth in buffaloes. No such lipid masses have been observed in the follicular epithelium of corresponding follicles in cattle (Guraya 1968) and other mammals (Guraya 1985). In growing follicle, theca cells, membrana granulosa and oocyte showed moderate reaction whereas basement membrane and antrum were weakly positive. Also the zona pellucida showed negligible amount of lipids. Similar results were observed in tertiary follicle. In atretic follicles, theca cells and granulosa cells showed moderate to strong reaction of sudanophilic lipids. Basement membrane and antrum showed weak reaction whereas oocyte was moderately positive. Like growing and tertiary follicle, zona pellucida of atretic follicle also contain negligible amount of lipids. Earlier histochemical studies have showed that with the start of atresia in pre-antral and antral follicles, their granulosa cells first hypertrophy and meanwhile granulosa cells and theca cells develop large number of sudanophilic lipid droplets (Guraya *et al.* 1994) which may give rise to masses of interstitial gland cells in the wall of atretic follicles

Connective tissue covering of corpus luteum showed weak to moderate reaction whereas luteal cells were strongly positive (Fig. 6). The abundance of sudanophilic lipid droplets has been reported in the luteal cells (Ahmed *et al.* 1984). It was suggested that such luteal cells actively function in the secretion of progesterone rather than in the storage of hormone precursor (cholesterol) (Guraya 1997). In medulla, rete ovarii showed weak to moderate reaction whereas blood vessels were moderately positive.

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