Immunohistochemical Examination Results

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Preparation : Ovarium
Staining : Immunohistochemical
Time of examination : 21 November 2021
Examinator : Djoko Legowo, M.Kes., drh.

Examination method

Expression of TNF-α

This histopathological examination was intended to determine TNF-α expression in the ovaries. The data of each sample was assessed semi-quantitatively according to the modified Remmele method (Novak et al., 2007), where the Remmele scale index (Immuno Reactive Score/IRS) is the result of multiplying the percentage score of immunoreactive cells with the color intensity score of immunoreactive cells (table 1). The data for each sample is the average IRS value observed in ten different fields of view at 100x and 400x magnification.

Table 1. The semi-quantitative RIS scale is the result of multiplying the percentage score of positive cells (A) with the color reaction intensity score (B), so IRS = (A x B)

A	B
Score 0 : no positive cells	Score 0 : no color reaction
Score 1 : Positive cells less than 10%	Score 1 : Weak color intensity
Score 2 : Positive cells between from 11% - 50%	Score 2 : Medium color intensity
Score 3 : Positive cells between from 51% - 80%	Score 3 : Strong color intensity
Score 4 : Positive cells more than 80%	

Surabaya, 21 November 2021
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(Djoko Legowo, M.Kes., drh)

Reference:
Novak M, Madej JA, Dziegeil P. 2007 Intensity of Cox 2 expression in Cell of Soft Tissue Fibrosarcomas in Dog As Related to Grade of Tumor malignation. Bull Vet inst Pulawy 51, 275-279

A. TNF-α Expression Scoring Results Data

Groups		Remmele scale index (IRS)										Mean	Additional information
		fields of view											
		1	2	3	4	5	6	7	8	9	10		
K-	1	6	6	3	6	3	3	3	2	3	3	3.8	
	2	6	4	6	6	6	3	3	6	6	3	4.9	
	3	4	3	4	4	6	3	6	2	3	3	3.8	
	4	9	6	3	6	6	6	6	6	3	3	5.4	
	5	9	9	6	6	9	6	9	9	6	6	7.5	
	6	4	6	6	6	6	6	9	4	4	6	5.7	
	7	4	4	6	6	3	3	6	3	4	4	4.3	
	8	8	12	9	6	6	6	6	6	6	6	7.1	
											Mean	5.3125	
K+	1	2	4	2	2	1	2	6	6	3	6	3.4	
	2	3	6	6	6	3	3	6	6	4	4	4.7	
	3	4	4	6	6	6	4	6	2	6	4	4.8	
	4	6	3	3	3	6	6	6	6	6	6	5.1	
	5	2	4	3	3	6	6	3	3	6	3	3.9	
	6	9	6	9	9	6	9	9	9	9	9	8.4	
	7	6	4	4	1	6	6	6	3	6	6	4.8	
	8	6	3	3	6	6	6	3	3	3	6	4.5	
											Mean	4.95	
P.1	1	6	6	4	6	6	6	4	6	6	6	5.6	
	2	4	4	4	6	6	2	6	6	6	4	4.8	
	3	9	6	2	6	6	6	6	6	6	6	5.9	
	4	6	4	4	6	6	6	3	4	2	2	4.3	
	5	4	6	4	4	4	4	4	2	6	4	4.2	
	6	6	4	2	4	6	4	6	6	6	9	5.3	
	7	2	2	2	2	2	2	2	4	4	4	2.6	
	8	4	4	4	6	9	4	9	6	6	6	5.8	
											Mean	4.8125	

P.2	**1**	6	6	6	6	6	6	6	6	6	6	6	
	2	3	3	3	6	6	6	6	4	2	6	4.5	
	3	6	9	6	6	3	6	6	6	3	3	5.4	
	4	4	4	8	8	8	9	8	8	8	6	7.1	
	5	3	3	3	3	3	3	1	3	6	6	3.4	
	6	3	3	3	3	1	3	3	1	1	3	2.4	
	7	3	6	6	6	9	6	6	6	6	3	5.7	
	8	6	6	3	6	2	3	3	2	2	2	3.5	
											Mean	**4.75**	

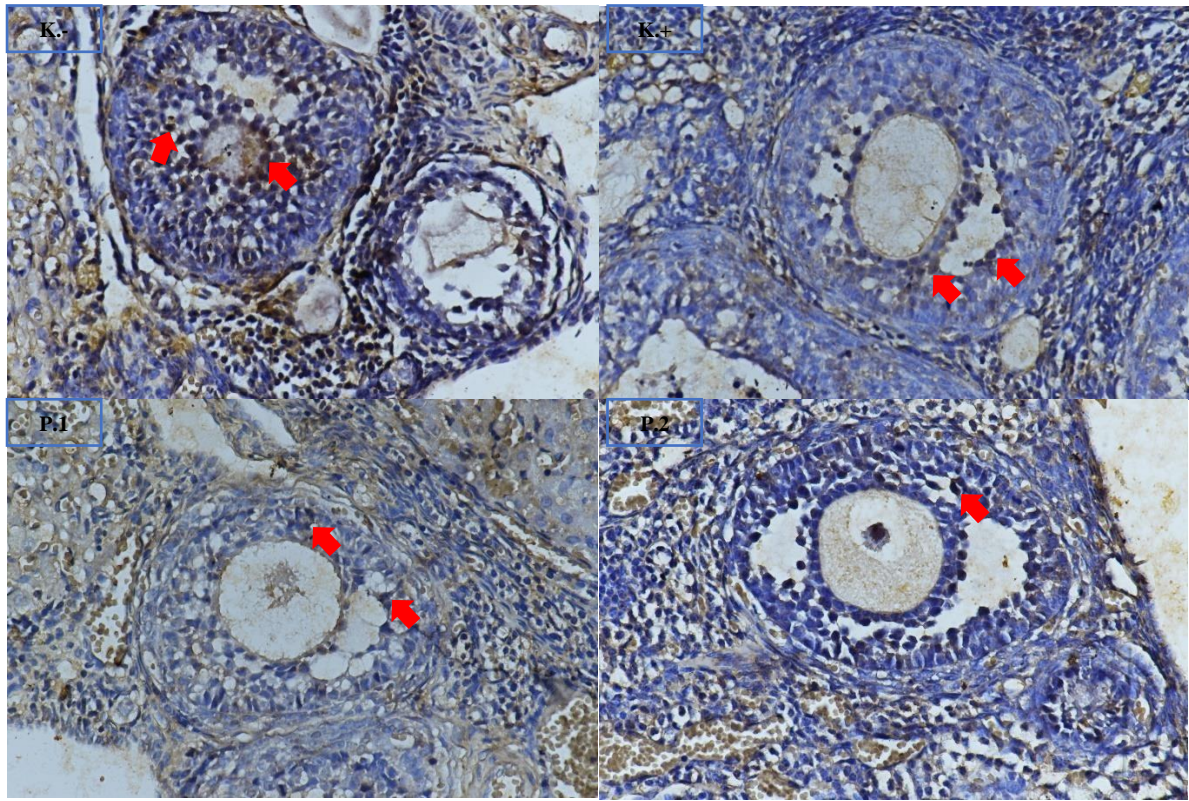**B. Histological Immunohistochemical Overview of TNF-α Expression**

Figure 1. Comparison of TNF- expression in the ovaries of the group (K-, K+P.1, P.2). The red arrow indicates the expression of TNF- s in the granulosa cells of the follicle which is indicated by the presence of a chromogen brown color. IHC. 400x