

ABSTRACT

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Title of Diploma Thesis: Influence of fixation time and type of fixative solution on immunohistochemical analysis in the detection of stem cells in endometrial tissue

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Background: The aim of this diploma thesis was to investigate the effect of the type of fixative solution and the length of fixation on immunohistochemistry.

Materials and methods: A total of five porcine and five bovine endometria obtained from hysterectomy specimens were included in this study. Immunohistochemical staining with progesterone receptors and SOX2 in endometrium fixed in five fixative solutions (Formol, Antigenfix, Greenfix, Bouin, Methacarn) were performed after 1,5; 8,5; 15,5; 29,5; 64,5 and 189,5 days of fixation. An automated immunostainer Ventana Benchmark Ultra was used for all immunohistochemical analysis.

Results: Expression of progesterone receptors was demonstrated in 92-87 % of glandular cells after 1,5-64,5 days and in 59 % of cells after 189,5 days of formalin fixation. The intensity of staining was strong, but after 189,5 days it was evaluated as being weak. In Antigenfix-fixed tissues, strong progesterone receptors expression was observed in 96,4 % of glandular cells after 1,5 days, in 77 % of cells after 29,5 days and in 72,6 % of cells with only moderate staining after 64,5 days of fixation. After 189,5 days, the immunoreaction was completely negative. In Bouin-fixed tissues, weak progesterone receptors expression was observed in 7,2 % of glandular cells after 1,5 days of fixation. Other time periods were negative. After only 1,5 days of fixation with Greenfix, 39 % of glandular cells showed weak progesterone receptors

expression. Immunohistochemical staining with progesterone receptors in methacarn-fixed tissues was completely negative over all time periods.

The intensity of staining for SOX2 after formalin fixation was determined to be extra-strong after 1,5 days, strong after 8,5; 15,5; 29,5 and 64,5 days and weak after 189,5 days of fixation. In the period of 1,5 to 189,5 days, an average of 98,4-94,8 % of glandular cells showed positivity with SOX2. The expression of SOX2 in Antigenfix-fixed tissues was the same over almost all time periods (positivity in more than 94 % of glandular cells). The intensity of staining was extra-strong after 1,5 and 8,5 days and strong after 15,5; 29,5; 64,5 and 189,5 days of fixation. After Bouin's fixation, moderate expression of SOX2 was detected in 96 % of glandular cells. Immunohistochemical staining was completely negative in tissues fixed with Greenfix and methacarn over any of the periods evaluated.

Conclusion: In our study, formol was the best fixative solution for immunohistochemistry. It proved to be the most suitable fixative for long-term and short-term fixation. Another very suitable fixative solution is Antigenfix, for which comparable results have been obtained with formol. Bouin's fixative solution can also be recommended for short-term fixation, but it is unsuitable for long-term fixation. Greenfix cannot be recommended for short-term fixation, it is completely unsuitable for long-term fixation. By far the worst fixative in our study was methacarn, which is completely unsuitable for immunohistochemistry (regardless of the time of fixation).

Key words: Immunohistochemistry, endometrium, fixative solution, progesterone receptors, SOX2