ORIGINAL RESEARCH

REFEREED

The Effects of Growth Factor on Testicular Germ Cell Apoptosis in the Stallion

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ABSTRACT

Apoptosis is necessary for both initiation and control of spermatogenesis; however, an increase in apoptosis can lead to subfertility/infertility in stallions, causing substantial financial loss in the equine industry. The ability of stem cell factor (SCF), leukemia-inhibiting factor (LIF), granulocyte–macrophage colony-stimulating factor (GM-CSF), and estradiol (E_2), alone or in combination, to prevent apoptosis of germ cells in short-term equine testicular cultures was examined. Testicular tissue was sectioned into approximately 2-mm cubes and placed in media-filled culture chambers. Concentrations of SCF (100 ng/mL), LIF (10 ng/mL), GM-CSF (5 ng/mL), and E_2 (10^{-9} mol/l) were added alone or in combination to each well. After 6 hours in culture, the tissue was fixed and immunohistochemically (terminal deoxynucleotidyl transferase-mediated nick-end labeling; TUNEL) stained for apoptosis detection. Apoptotic cells per 100 Sertoli cell nuclei within seminiferous tubules were counted until the 500th Sertoli cell nuclei was reached. This counting procedure was used for each slide. An analysis of variance (ANOVA) with a Tukey's test was used to compare apoptotic rates. In comparison with the control, GM-CSF alone lowered apoptosis by 34.77%. GM-CSF–treated tissue combined with SCF and LIF as well as GM-CSF combined with SCF, LIF, and E_2 reduced apoptosis when compared with the control (37.45% and 44.40%, respectively) or other treatment combinations. GM-CSF alone reduced apoptosis; results suggest possible synergy for the combinations of SCF and LIF with GM-CSF and for E_2 with SCF, LIF, and GM-CSF.

Keywords: Apoptosis; Stallion; Growth factors; Granulocyte–macrophage colony-stimulating factor (GM-CSF); Spermatogonia

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INTRODUCTION

Stallion subfertility/infertility is common[1] and may cause substantial financial loss in the equine industry.[2-4] Among the possible abnormalities or causes of germ cell loss may be an increase in apoptosis at the germ-cell level. During normal spermatogenesis, up to one-half of all germ cells never differentiate into mature sperm because of apoptotic-related mechanisms.[5] Increased apoptosis can reduce sperm count,[5,6] leading to subfertility.

The growth factors used in this study have known roles in spermatogenesis and apoptosis. In the human, addition of 17β-estradiol (10^{-9} mol/l) inhibited germ cell apoptosis and reduced apoptosis in spermatogenic cells in vitro.[7] In mice, reduced expression of stem cell factor (SCF) increased germ cell apoptosis,[8] and leukemia-inhibiting factor (LIF) acted directly on primordial germ cells to suppress apoptosis.[9,10] Both SCF and LIF act synergistically with colony-stimulating factors such as granulocyte–macrophage colony-stimulating factor (GM-CSF).[10,11] In a test conducted on normal human hematopoietic cells, Leary et al[10] suggest that LIF/DIA (differentiation-inhibiting activity) along with G-CSF (granulocyte colony-stimulating factor) may be involved in early hematopoietic stem cell regulation. Also, in a study conducted by Martin et al[11] using genomic clones of human SCF, SCF showed strong synergistic effects with colony-stimulating factors. GM-CSF recently has been shown to cross the blood–testis barrier intact,[12] and it is able to promote the survival of porcine type A spermatogonial cells.[13]

The objective of this study was to determine whether the growth factors LIF, SCF, E_2, and GM-CSF alone or in combination, might lower the incidence of apoptosis in stallion testicular germ cells, thereby identifying a possible treatment for this condition.

MATERIALS AND METHODS

Testes Collection, Culture, and Growth Factor Administration

Two stallions aged 5 and 7 years and of known reproductive soundness (determined by daily sperm output, motility, and concentration over a 3-month period) were castrated under general anesthesia. A section of testes from each horse was wrapped in aluminum foil and put on ice (for no more than 10 minutes). The testicular

Table 1. Mean number of apoptotic cells per 100 Sertoli cell nuclei and the percentage difference of each treatment versus the control

Growth Factor Treatments										
	Control	E2	SCF	LIF	GMCSF*	SCF+ LIF	SCF+ GMCSF	LIF+ GMCSF	SCF+LIF+ GMCSF*	E2+SCF+ LIF+GMCSF*
C1 mean	18.94	15.20	23.42	22.71	13.06	18.41	18.07	17.31	8.89	10.14
C2 mean	24.06	28.39	27.47	24.45	14.99	27.93	15.56	18.53	18.01	13.78
% Difference from control		−1.3	−18.33	−9.69	34.77	−7.77	21.80	16.66	37.45	44.40

The first horse was designated as culture 1 (C1) and the second culture 2 (C2). Each treatment had two wells, with a piece of tissue in each well. The mean number of apoptotic cells per 100 Sertoli cell nuclei per treatment is shown. The last row is the overall percent difference of each treatment compared with the control. A negative number signifies an increase and a positive number a decrease.

* $P < .05$.

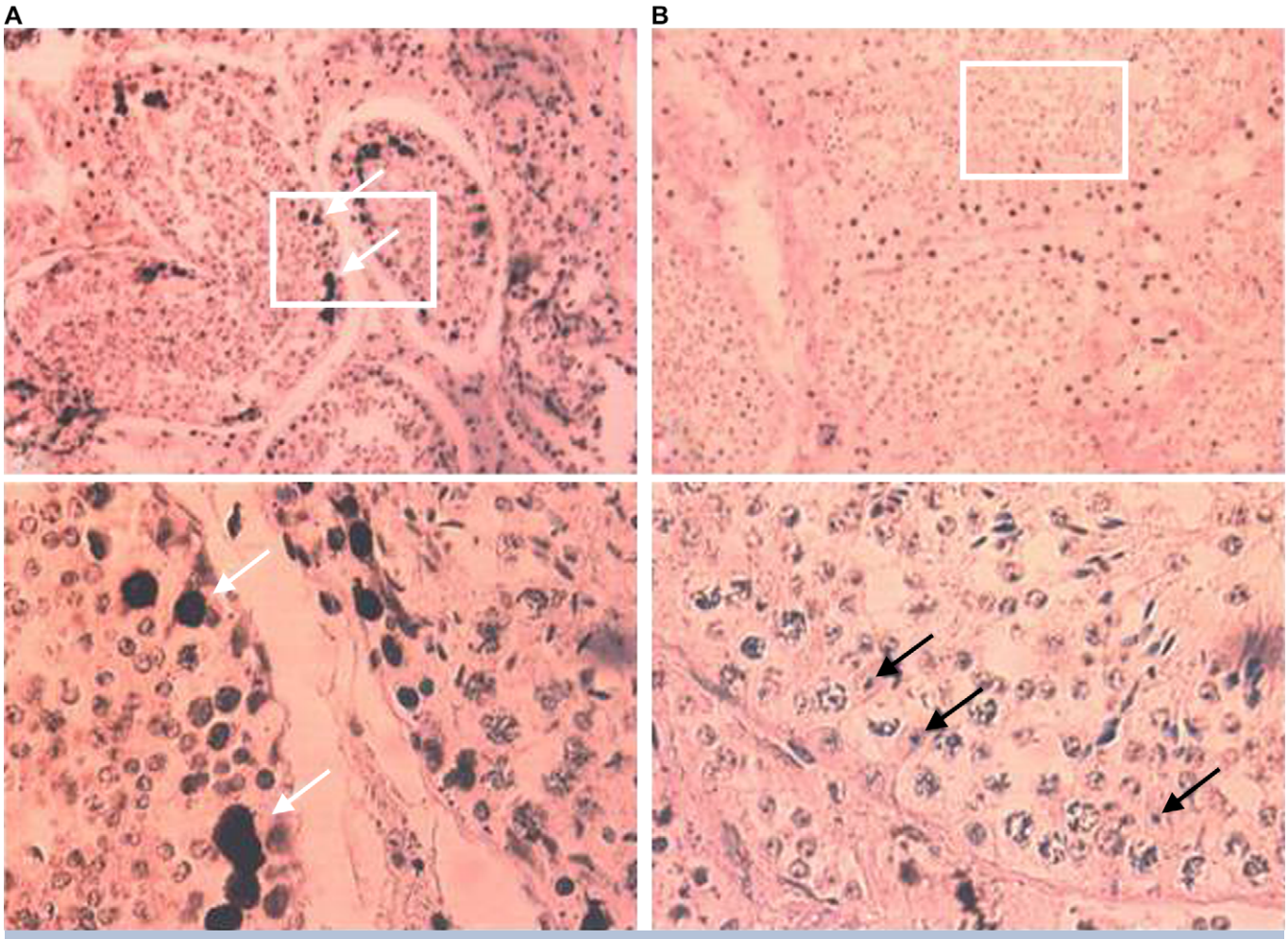

Figure 1. Immunohistochemical localization of apoptotic cells within the seminiferous epithelium. Labeled apoptotic cells are shown in A, whereas few or no labeled apoptotic cells within the seminiferous epithelium are shown in B.

samples were sectioned into approximately 2-mm cubes and placed into culture chambers fitted with bicameral cell culture inserts (Becton Dickinson Labware, Franklin Lakes, NJ). They were filled with media (Hanks F12/DMEM 1:1) supplemented with 4 mmol/l glutamine,[14] 4 mmol/l sodium lactate, 1 mmol/l sodium pyruvate,[15] and 50 µg/ml ascorbic acid as an antioxidant[16] and incubated for 6 hours[17] at 32°C at 5% CO_2.

Dosages

Media only was used as the control. SCF (100 ng/ml),[13] LIF (10 ng/ml),[18] GM-CSF (5 ng/ml),[13] or E_2 (10^{-9} M)[7]