BOVINE COLOSTRUM IMPROVES BONE MICROSTRUCTURE IN OVARIECTOMIZED AND ORCHIDECTOMY RATS VIA VEGFA SIGNALING

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PURPOSE

Research has shown that components of bovine colostrum (BC), as lactoferrin, induce bone anabolic effects. However, the effects of BC supplementation as a whole in bone microarchitecture and the mechanisms through which BC may induce bone anabolic effects is relatively unclear. The aim of this study was to evaluate the effects of different BC doses supplementation in ovariectomized (OVX) and orchidectomy (ORX) rat-models and to identify the pathways that may mediate the action of BC on bone.

METHODS

After baseline measurements of bone microarchitecture (μCT), twenty-seven weeks-old female (OVX, n=32) and male (ORX, n=32) Wistar Han rats were randomly assigned to the following groups: a) placebo b) BC dose 1 (BC1; 0.5g/day/females; 1.0g/day/males), c) BC dose 2 (BC2; 1g/day/females; 1.5g/day/males) and d) BC dose 3 (BC3; 1.5g/day/females; 2g/day/males). After 4 months of supplementation, bone microarchitecture was re-assessed. Gene expression of VEGFA, FGF2, OPG, RANKL and RANK were measured by qRT-PCR. The study was approved by the National Ethics Committee for the Use of Animals in Research.

Figure 2. Project timeline

Pre-Intervention	4 Months Bovine Colostrum Supplementation	Post-Intervention
1. Bone formation and resorption markers: alkaline phosphatase, osteocalcin, deoxypyridinoline, CTX 2. Micro-CT	BC1 (OVX): 0.5g/day/females; 1.0g/day/males BC2 (OVX): 1g/day/females; 1.5g/day/males BC3 (OVX): 1.5g/day/females; 2g/day/males Placebo (OVX)	1. Bone formation and resorption markers: alkaline phosphatase, osteocalcin, deoxypyridinoline, CTX 2. Micro-CT 3. Mechanical testing 4. Gene expression: VEGFA, FGF2, OPG, RANKL, RANK

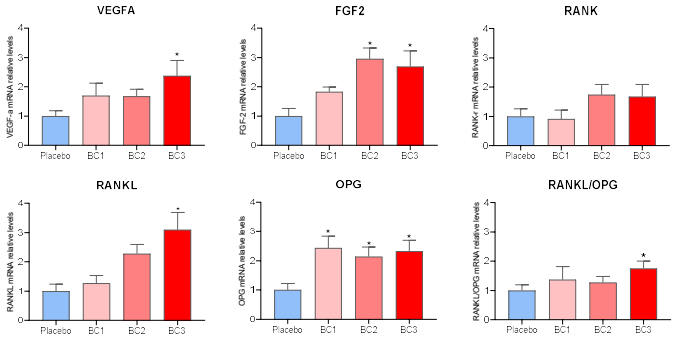

Figure 1. The qPCR analysis for VEGFA, FGF2, RANK, OPG, RANKL and the RANKL/OPG ratio.
Data are mean ± SD; *p<0.05, significantly different from placebo.

RESULTS

Regarding male rats, BC1 significantly improved bone microarchitecture, as these rats not only revealed significantly less cortical porosity (41.9%, p<0.05) and cortical pore size (25.7%, p<0.05) compared to placebo group, but also significantly increased cortical volume (89.7%, p<0.05), cortical BMD (134.9%, p<0.01), trabecular thickness (37.3%, p<0.01), trabecular volume (24.6%, p<0.001) and trabecular BMD (7.5%, p<0.01). BC2 induced the same effects as dose 1 for male rats, and significantly reduced trabecular porosity (8.1%, p<0.01), while dose 3 reduced trabecular separation (29.3%, p<0.05). As for female rats, BC1 did not induce positive effects on bone microarchitecture. However, BC2 and BC3 decreased cortical porosity (placebo: 65.75±4.22; dose 2: 25.16±8.83; dose 3: 25.22±8.54%, p<0.01) and improved trabecular thickness (placebo: 12.22±0.99; dose 2: 21.11±3.28; dose 3: 18.39±2.45μm, p<0.01) compared to placebo. BC1 significantly increased only the mRNA expression of OPG (1.97±1.29, p<0.05). BC2 caused a significant increase in the mRNA levels of FGF2 (2.95±1.36, p<0.05) and OPG genes (2.14±1.31, p<0.05). Finally, BC3 induced a significant increase in mRNA expression of VEGFA (2.37±1.83, p<0.05), RANKL (3.10±2.27, p<0.05) and in the RANKL/OPG ratio (1.75±0.99, p<0.05). None of the doses affected the local RANK gene expression (p>0.05).

Table 1. Post-supplementation μCT results for the placebo and BC supplementation groups (BC1, BC2, BC3) – Male and Female rats

	Post-intervention – Male rats				Post-intervention – Female rats			
Analyses parameter	**Placebo**	**BC1 (1.0g/day)**	**BC2 (1.5g/day)**	**BC3 (2.0g/day)**	**Placebo**	**BC1 (0.5g/day)**	**BC2 (1.0g/day)**	**BC3 (1.5g/day)**
Cortical porosity (%)	68.12 ± 5.62	**39.56 ± 15.47***	**25.51 ± 13.63*****	**25.92 ± 7.39****	65.75 ± 4.22	**68.03 ± 14.32***	**25.16 ± 8.83****	**25.22 ± 8.54****
Cortical pore size (μm)	33.32 ± 6.61	**24.75 ± 4.21***	**18.99 ± 6.32****	**16.71 ± 3.33****	28.85 ± 6.18	**37.44 ± 7.01***	**23.36 ± 2.99***	**22.57 ± 4.98***
Cortical volume (% BV/TV)	31.87 ± 5.62	**60.44 ± 15.47***	**74.49 ± 13.63*****	**74.09 ± 7.40****	34.25 ± 4.22	**31.98 ± 14.31***	**74.84 ± 8.83****	**74.78 ± 8.54****
Cortical BMD (g/cm³)	1.09 ± 0.04	**2.56 ± 0.91****	**3.17 ± 0.44*****	**2.93 ± 0.37****	1.13 ± 0.08	**1.29 ± 0.63***	**2.33 ± 0.73***	**2.83 ± 0.31****
Cortical BMC (g)	95.91 ± 7.32	**72.52 ± 13.58****	**74.53 ± 23.13****	**78.93 ± 10.89****	98.14 ± 1.20	94.24 ± 9.19	74.99 ± 17.55	**71.78 ± 14.76****
Trabecular porosity (%)	91.62 ± 2.55	89.56±3.13	**84.23 ± 4.26****	**84.92 ± 4.22****	85.01 ± 6.37	**92.47 ± 3.85***	86.62 ± 4.80	85.26 ± 2.24
Trabecular separation (μm)	110.14 ± 33.50	150.04 ± 51.30	86.44 ± 38.52	**77.91 ± 22.43***	85.28 ± 39.01	**153.84 ± 79.62***	145.79 ± 91.25	104.57 ± 47.40
Trabecular thickness (μm)	11.25 ± 0.76	**15.45 ± 4.42****	**17.91 ± 3.66*****	**16.78 ± 1.55****	12.22 ± 0.99	12.64 ± 3.64	**21.11 ± 3.28****	**18.39 ± 2.45****
Trabecular volume (% BV/TV)	8.38 ± 2.55	**10.44 ± 3.13*****	**15.77 ± 4.26****	**15.08 ± 4.22****	14.99 ± 6.37	**7.53 ± 3.85***	13.75 ± 4.93	14.74 ± 2.24
Trabecular BMD (g/cm³)	1.07 ± 0.34	**1.15 ± 0.20****	**1.29 ± 0.22*****	**1.17 ± 0.25****	1.14 ± 0.11	1.07 ± 0.09	0.97 ± 0.28	**1.22 ± 0.12****
Trabecular BMC (g)	98.74 ± 2.56	66.25 ± 17.12	78.61 ± 10.60	**74.19 ± 10.19****	99.12 ± 0.41	**94.98 ± 12.48***	71.59 ± 19.30	72.71 ± 13.40

Values are mean ± SD; *p<0.05; **p<0.01; ***p<0.001, significant different from placebo; BMD = bone mineral density; BMC = bone mineral content

CONCLUSIONS

BC preserves bone mass of OVX and ORX rats by stimulating bone formation and by restraining bone resorption in a dose-dependent manner. Indeed, as bone is a high vascularized organ and VEGFA and FGF2 play important roles in vascular development and angiogenesis, it seems that VEGFA and FGF2 are influencing skeletal development and osteogenesis in OVX and ORX rats. In turn, BC appears to act on the RANKL/RANK/OPG signaling pathway as well, a great regulator of bone resorption.

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