Effect of different housing conditions on several indices of blood redox status and on reproductive performance in buffalo cows

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ABSTRACT: The aim of the present study was to examine the effect of two different housing conditions on blood and follicular redox status and on reproductive performance of buffalo cows. Fifty buffalo cows were housed at a density of 13.3 m^2/head, and fifty were housed at 27.0 m^2/head. Forty buffaloes for each group were inseminated, and blood samples were collected 45 days after AI. Ten buffaloes, for each group, were not inseminated, and follicular fluids and blood samples were collected at the time of ovulation. Titres of retinol, α-tocopherol, ascorbate, N-tyrosine and protein bound carbonyls were measured. No differences were found between the two groups. Redox status of pregnant, not pregnant and cows with embryo mortality was evaluated. We found no significant differences among the groups, thus suggesting that the outcome of fertilization might not depend only on the blood parameters evaluated. Our results demonstrate that the two different housing conditions do not affect plasma and follicular redox status.

Key words: Oxidative stress, Reproduction, Housing condition.

INTRODUCTION - The use of systems which reduce the effect of stress, especially high temperature stress, and mainly the use of systems which protect the buffalo from direct sunlight, improves reproductive performance (Campanile and Balestrieri, 2002). In fact, buffaloes benefit from the possibility of dipping in water, as showed by an improvement in their reproductive activity (Di Palo et al., 2001). Deficiencies of natural protective substances or excessive exposure to stimulators of ROS production may result in oxidative stress, which occurs when prooxidants exceed the capacity of antioxidants (Halliwell and Gutteridge, 1999). Antioxidants prevent oxidative stress in healthy ovarian follicles, where the maturation and the quality of oocyte depend on the function of the granulosa cells. Cell death and atresia occur in the follicle under oxidative stress (Dharmarajan et al., 1999). The aim of this work was to evaluate the effect of space availability on blood and follicular redox status, and on reproductive performance.

MATERIAL AND METHODS - The trial was carried out between January and April 2007 on 100 buffaloes equally divided on the basis of days in milk (DIM) in two groups space availability: Group S (DIM=138.0 ± 13.0) and Group NS (DIM=133.4 ± 12.0). Buffaloes were housed at a density of 13.3 m^2/head and 27.0 m^2/head in Group S and NS, respectively. After 60 days, all animals were synchronized by the Ovsynch-TAI Program and 40 buffaloes for each group were bred by AI 16 h and 40 h after the second injection of GnRH agonist. Ten buffaloes for each group were not bred, and underwent transvaginal follicular aspiration and blood sampling at the time of ovulation. Plasma samples, obtained by centrifugation (500 *g*; 15 min; 4°C), and follicular fluids were immediately analyzed. On day 26 after AI, buffaloes were monitored by ultrasound to verify the presence of a pregnancy. The cyclic ovarian status of the buffaloes and luteal function were evaluated by measuring progesterone (P_4) concentrations by RIA in two blood samples collected on days 10 and 20 after AI. Blood P_4 concentrations >1.5 ng/ml were considered to be indicative of the presence of an active corpus luteum. On the day of AI and 10 and 20 days after AI blood samples were collected to analyze estradiol (E_2). Pregnancy diagnosis was confirmed on day 45 after AI, using ultrasonography, and blood samples were again collected. Buffaloes pregnant on day 26 but not on day 45 were considered to have undergone embryonic mortality. Follicular fluid and plasma of not inseminated buffaloes and plasma of inseminated buffaloes were used for titration of liposoluble (retinol and α-tocopherol) and hydrophilic (ascorbate) antioxidants, and protein oxidation markers (nitro-tyrosine and protein-bound carbonyls). In particular, plasma levels of ascorbate, retinol and α-tocopherol were measured as previously described (Spagnuolo et al., 2001); nitrated protein concentration (N-Tyr) in plasma samples was titrated by ELISA, and the assay was performed essentially according to Spagnuolo et al. (2001). Data were reported as nmoles of N-Tyr per mg of protein. Protein carbonyls (PC) in plasma samples were titrated by ELISA, essentially according to Buss et al., (1997). The samples for determining PC and N-Tyr concentration were processed in triplicate. The titration of α-tocopherol, retinol, and ascorbate was carried out on duplicates. Values are expressed as mean ± SEM. The software MINITAB was used to perform analysis of variance, t-test, and to calculate significance.

RESULTS AND CONCLUSIONS - No differences were found between the two groups in ascorbate, retinol and α-tocopherol plasma levels (Table 1). Titres of plasma N-Tyr and PC did not differ between S and NS group. (Table 1). As shown in table 2, in the ovarian follicular fluids the analysed indices of redox status did not differ between S and NS group. It is worth to mention that the antioxidant defence system plays a key role in preventing apoptosis and atresia, thus preserving steroidogenic function of granulosa cells (Cassano et al., 1999). As there were no significant differences between S and NS cows, the data were also analysed altogether, to compare redox status of pregnant (P), not pregnant (NP) and cows with embryo mortality (EM). Plasma levels of retinol, α-tocopherol and ascorbate, as well as titres of protein oxidation markers (N-Tyr and PC), did not differ between P and NP cows. This result suggests that metabolic processes and endocrine changes associated with pregnancy do not affect the indices of redox status here analysed. We also found no significant differences between P and EM buffaloes (data not in table), thus suggesting that the outcome of fertilization might be not dependent only on the blood parameters here evaluated.

Table 1. Several markers of redox status in plasma in two experimental groups.

	NS	S
n.	50	50
Retinol (μg/ml)	0,509 ± 0,013	0,492 ± 0,015
α-Tocopherol (μg/ml)	1,627 ± 0,088	1,819 ± 0,119
Ascorbate (μM)	5,643 ± 0,286	5,623 ± 0,304
PC (nmoesl/mgP)	7,594 ± 0,671	8,125 ± 0,718
N-Tyr (nmoles/mgP)	1,269 ± 0,097	1,322 ± 0,089

Table 2. Several markers of redox status in follicular fluid in two experimental groups.

	S	NS
n.	7	4
Retinol (μg/ml)	0,105 ± 0,026	0,156 ± 0,050
α-Tocopherol (μg/ml)	0,337 ± 0,088	0,302 ± 0,105
Ascorbate (μM)	4.774 ± 0,200	4.303 ± 0,048
PC (nmoles/mgP)	20,02 ± 5,977	7,591 ± 3,905
N-Tyr (nmoles/mgP)	16,11 ± 6,176	6,035 ± 2,173

Plasma levels of P_4 were significantly higher in P than in both NP and EM buffaloes, at days 20 and 25. Embryonic mortality in buffaloes are due primarily to reduced secretion of P_4 by the corpus luteum linked with a reduced capacity of the developing embryo to secrete IFNt interferon at threshold amounts necessary to prevent luteolysis (Campanile, 2005). No differences were found in P_4 plasma levels between not pregnant and cows with embryo mortality. E_2 plasma levels did not differ between the different reproductive status considered (Table 3). These results demonstrate that the two different housing conditions do not

Table 3. E_2 (pg/ml) and P_4 (ng/ml) plasma levels in P, NP and EM buffaloes on different days after AI.

	P	NP	EM
n.	50	22	8
E_2 0	21.9 ± 0.9	20.6 ± 0.9	18.9 ± 1.2
E_2 10	18.9 ± 0.7	18.1 ± 0.9	17.0 ± 1.5
E_2 20	16.6 ± 0.5	16.4 ± 0.706	17.1 ± 1.4
E_2 25	15.5 ± 0.1	15.8 ± 0.1	16.0 ± 0.2
P_4 10	3.0 ± 0.1	2.4 ± 0.3	2.0 ± 0.7
P_4 20	3.7 ± 0.1 Aa	1.8 ± 0.4 B	2.0 ± 0.7 b
P_4 25	4.0 ± 0.1 A	1.0 ± 0.3 B	2.2 ± 0.7 B

A vs. B = P<0.01; a vs. b = P <0.05

affect plasma and follicular redox status, thus suggesting that stabulation of buffalo cows at a density of 13.3 m^2/head might be used without compromising of animal welfare and of follicular development. Furthermore, embryonic mortality seems to depend on progesterone plasma level, but not on oxidative stress.

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