

***In vitro* buffalo embryo production under heat stress: Role of granulosa cells co-culture as a monolayer**

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Abstract

Effect of heat shock and co-culture of monolayer granulosa cells (GCs) on *in vitro* embryo developmental competence in Egyptian buffalo was the aim of the present study. Cumulus oocytes complexes (COCs) were collected from ovaries ($n = 448$) of cyclic slaughtered buffalo cows. Good quality immature oocytes ($n = 1512$) were subjected to *in vitro* maturation and fertilization. Post 18-22 h of fertilization, presumptive embryos were randomly assigned into four groups: (G1) No heat shock (38.5°C), (G2) Heat shock (40.5°C) and (G3) Co-culture with GCs monolayer and heat shock or (G4) Co-culture with GCs monolayer and no heat shock. Heat-shocked embryos were exposed to temperature of 40.5°C for the first two hours of culture before exposing to 38.5°C afterward up to the end of culture period (8 days). The data were analyzed by General Linear Univariate model using SPSS.

Oocyte expansion rate was $90.8 \pm 1.1\%$. This percentage is higher than nuclear maturation rate (oocytes at Telophase and Metaphase II) as detected by aceto-orcein staining by about 17%. Cleavage rate as recorded at day 3 post fertilization (day 0) was higher ($p \leq 0.05$) for G1 and G4 ($71.1 \pm 10.5\%$ and $70.5 \pm 7.9\%$ with GCs, respectively). On the other hand, cleavage rate was dramatically decreased ($P \leq 0.05$) for embryos of G2 ($43.7 \pm 7.0\%$) compared with that of G3 ($80.2 \pm 7.0\%$). In addition, embryos of G3 showed approximately the same rate of developed embryos (Morula and blastocyst stages at day 8 of culture) as of G1 ($50.9 \pm 5.3\%$ and $51.7 \pm 7.9\%$, respectively). In conclusion, using co-culture of GCs as a monolayer enhances cleavage rate and subsequent embryo development of buffalo embryos under heat stress condition.

Keywords: *in vitro* embryo production, Heat stress, Co-culture, Buffalo