Available online at www.sciencedirect.com

ScienceDirect

Theriogenology 72 (2009) 978–985

Theriogenology

www.theriojournal.com

Factors affecting laboratory production of buffalo embryos: A meta-analysis

K.P. Suresh, S. Nandi [*], S. Mondal

National Institute of Animal Nutrition and Physiology, Adugodi, Bangalore, India

Received 31 January 2009; received in revised form 25 June 2009; accepted 28 June 2009

Abstract

In vitro fertilization (IVF) provides an excellent and inexpensive source of embryos for carrying out basic research on developmental physiology, farm animal breeding, and for commercial applications. Meta-analysis of the results from different publications rather than a narrative review may provide a current status of this technology in buffalo (Bubalus bubalis). In order to gain an idea of the factors affecting the IVF in buffalo, a review of the various studies conducted on buffalo IVF and a meta-analysis of their findings was undertaken. More than 100 articles published from 1991 to 2008 were searched, and results were subjected to meta-analysis to determine the treatment variations without any bias. Thirty factors affecting in vitro embryo production in buffalo were considered. Initially, both fixed- and random-effect models were used. We did not observe any heterogeneity between the studies. Thereafter, all the studies were pooled using the fixed-effect model for analysis. Our analysis suggested that good buffalo oocytes with more than three to five cumulus layers recovered from large-sized follicles in cold seasons when cultured in TCM-199 supplemented with serum, follicle-stimulating hormone, and cysteamine resulted in maximum maturation rate and subsequent embryonic development after insemination. The values obtained in the current study may be considered for a simulation model in establishing a cost-effective suitable method for buffalo IVF in further planned research.

Keywords: In vitro fertilization (IVF); Buffalo; Meta-analysis

---

1. Introduction

Inherent reproductive problems limit the productivity of buffalo, an important species in India in terms of milk and meat production as well as draft. Assisted reproductive technologies such as artificial insemination (AI), superovulation, in vitro fertilization (IVF), and embryo transfer (ET) have been introduced to overcome these problems, to increase the number of offspring from selected females, and to reduce the generation intervals in buffalo. Laboratory production of embryos (IVF technology) provides an excellent and inexpensive source of embryos for carrying on basic research in developmental physiology, farm animal breeding, and for commercial application of the emerging biotechniques like cloning and transgenesis. During the past two decades, considerable advancements have been made as a result of continuous scientific effort. However, various previous reviews [1–3] and recent studies suggest that the rate of transferable embryo yield remains at a plateau [4–7]. There are many laboratory-to-laboratory variations, and there is a need to analyze the conditions, protocols, and factors that affect the successes rate of IVF in buffalo. Hence, a meta-analysis was performed using evidence-based research to study the factors affecting the success rates in terms of maturation rate, fertilization rate, and

---

[*] Corresponding author. Tel.: +91 80 25711164; fax: +91 80 2571420.
E-mail address: snandi71@yahoo.co.in (S. Nandi).

0093-691X/$ – see front matter © 2009 Published by Elsevier Inc.
doi:10.1016/j.theriogenology.2009.06.017

embryo yield in buffalo obtained in various laboratories. The results may be useful to analyze the current status of IVF in buffaloes and to throw some lights on the future direction of research in this field. This study may identify the factors causing the inconsistencies between previous studies and thus can play a key role in planning new studies.

2. Materials and methods

Articles published on in vitro production of embryos in buffalo (Bubalus bubalis) were sought using the on-line journal databases Web of Science (ISI), CAB (CABI Publishing), and VET CDs. Articles were also found by cross-referencing citations in retrieved articles. No unpublished study was considered. About 109 publications were searched, and results were subjected to meta-analysis to determine the treatment variations without any bias. All authors of this article independently abstracted study reports. The investigators of the original publications were contacted if required information was not available. Care was taken to reduce the publication bias. Thirty factors (Table 1) affecting in vitro embryo production in buffalo were considered. Wherever data was available from two articles, the meta-analysis was performed. Meta-analysis was performed on the in vitro maturation rates, cleavage rates, and embryo yield in buffalo influenced by these factors.

Given a vast quantity of heterogeneous literature, the type of items that were collected include the characteristics of the report (such as author, year, and source), the study itself, research design (experimental or observational, treatment assignment mechanism or sampling mechanism, attrition rate or nonresponse rate), and the effect size (sample size, nature of outcome, estimates, and standard error). These factors are given the first half of Table 1. Meta-analysis was performed using the methodology described [8–10]. The detailed methodologies used in the current were as described in the following sections.

2.1. Test of significance of homogeneity

The standard test for the equality of k variances was carried out. The k study-specific summary statistics shared a common mean θ. A statistical test for the homogeneity of study means was equivalent to testing,

H_0: $\theta = \theta_1 = \theta_2 = \ldots\ldots\theta_k$ against

H_1: At least one θ_i is different.

Under H_0, for large sample sizes, $Q_w = \sum_1^k w_i(y_i - \theta_{\hat{\text{MLE}}})$ follows chi-square with $(k-1)$ degrees of freedom, where $\theta_{\hat{\text{MLE}}} = \frac{\sum w_i y_i}{\sum w_i}$ and $w_i = \frac{1}{s_i^2}$.

2.2. Fixed-effect model

The inverse-variance method (I-V method) was used to pool either binary, continuous, or correlation data. The effect sizes were combined to give a pooled estimate (denoted by θ) by calculating weighted average of the treatment effects from the individual studies as follows:

$$\theta_{\text{I-V}} = \frac{\sum w_i \theta_i}{\sum w_i}$$

where the weights w_i were calculated as

$$w_i = \frac{1}{\text{SE}(\theta_i)^2},$$

that is, the weight for the ith study was equal to its precision of the estimate.

The standard error of $\theta_{\text{I-V}}$ was given by,

$$\text{SE}(\theta_{\text{I-V}}) = \frac{1}{\sqrt{\sum w_i}}.$$

The heterogeneity statistic (denoted by Q_w) was given by

$$Q_w = \sum w_i(\theta_i - \theta_{\text{IV}})^2.$$

The Q_w followed chi-square distribution with $(k - 1)$ degrees of freedom, where k was the number of studies included in the meta-analysis.

2.3. Random-effect model

Under the random-effect model, the assumption of common effect was relaxed, and the effect size θ_i was assumed to have a normal distribution with mean θ and variance τ^2. The usual pooled estimate of random effect model (DL) estimate for τ^2 was given by

$$\tau^2 = \frac{Q_w - (k-1)}{\sum w_i - \frac{\sum w_i^2}{\sum w_i}}$$

where Q_w was the heterogeneity statistic, and the weights w_i were calculated as described earlier, and k is the number of studies. The τ^2 was set to zero if $Q_w < (k - 1)$. In this approach, the weights for each study effect size $w_i^{'}$ were as given below:

$$w_i^{'} = \frac{1}{\text{SE}(\theta_i)^2 + \tau^2}.$$