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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

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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).

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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,

$Q_w = \sum_1^k w_i(y_i - \theta_{\hat{MLE}})$ follows chi-square with $(k-1)$ degrees of freedom, where $\theta_{\hat{MLE}} = \frac{\sum w_i y_i}{\sum w_i}$ 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:

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

Table 1
The maturation, fertilization, and morulae/blastocyst yield after subjecting the data to meta-analysis.

Serial no.	Factors	References	Maturation rate	Cleavage rate	Morulae/blastocysts yield
1	Serum	11–33	80.56 ± 0.06 (3127)	51.79 ± 0.14 (2632)	16.08 ± 0.09 (2310)
2	FSH+LH+E	13,26,31,34–46	71.73 ± 0.16 (1662)	53.21 ± 0.14 (2762)	18.27 ± 0.10 (2639)
3	FSH	13–16,20,30,32,47–51	77.09 ± 0.23 (1779)	43.64 ± 0.12 (1766)	19.51 ± 0.17 (1384)
4	FSH+E2	13,19,52–54	62.68 ± 0.47 (521)	27.96 ± 0.60 (314)	14.0.3 ± 0.49 (314)
5	Estradiol	13,39	80.62 ± 0.92 (387)	*	*
6	PMSG	11,14,15,39,48,55–59	86.08 ± 0.10 (1288)	52.74 ± 0.15 (969)	17.16 ± 0.27 (908)
7	Follicular fluid	11,12,23,51,60–65	75.6 ± 0.16 (1997)	40.68 ± 0.21 (1700)	12.61 ± 0.19 (1700)
8	Growth factor: EGF	41,53,66–69	79.32 ± 0.26 (1183)	42.50 ± 0.44 (872)	15.59 ± 0.41 (748)
9	Growth factor: IGF	49,53,66,70	78.06 ± 0.44 (587)	41.28 ± 0.62 (496)	19.28 ± 0.76 (374)
10	BSA	69,71–73	35.33 ± 0.31 (680)	7.34 ± 0.47 (370)	2.22 ± 0.13 (196)
11	Cysteamine	5,34,38,42,74	86.27 ± 1.23 (579)	58.67 ± 0.31 (1181)	26.98 ± 0.19 (1181)
12	β-mercaptoethanol	75–77	70.54 ± 1.71 (594)	24.29 ± 2.14 (472)	6.53 ± 0.39 (472)
13	Protein fraction supplement	71,72	84.26 ± 0.80 (227)	*	*
14	Monolayer	56,60,78	70.75 ± 1.27 (187)	*	*
15	TCM-199	13,26,35,37,48,78–81	80.05 ± 0.04 (790)	42.47 ± 0.09 (391)	18.13 ± 0.59 (301)
16	Ham's F-10	13,26,35,37,48,79–81	52.31 ± 0.10 (647)	38.67 ± 0.20 (187)	*
17	MEM	35,48,82	72.45 ± 0.08 (202)	*	*
18	Defined maturation medium	16,41,68,70,83,84	82.88 ± 0.48 (726)	48.57 ± 0.61 (697)	15.49 ± 0.42 (761)
19 (i)	Cumulus layers (good)	14,22,28,36,48,85–89,14	69.49 ± 0.28 (948)	59.95 ± 0.35 (783)	25.79 ± 0.29 (744)
19 (ii)	Cumulus layers (average)	14,22,28,36,48,85–89,41	35.24 ± 0.23 (988)	40.67 ± 0.40 (855)	4.76 ± 0.14 (779)
20	IVF media (BO)	22,24,43,68,90	NA	46.21 ± 0.41 (860)	14.6 ± 0.33 (750)
21	IVF media (TALP)	18,43,90,91	NA	38.23 ± 0.55 (450)	22.90 ± 0.72 (240)
22	Caffeine	14,25,28,87,88	NA	35.36 ± 0.29 (612)	20.92 ± 0.62 (346)
23	Theophylline	43,92	NA	29.43 ± 1.08† (180)	*
24	Sperm concentration (low)	43,87,91,93	NA	32.18 ± 0.17 (347)	14.32 ± 0.54 (223)
25	Sperm concentration (moderate)	28,87,88,91	NA	34.95 ± 0.45 (271)	*
26	Sperm concentration (high)	25,55,87,92	NA	51.68 ± 0.61 (640)	20.60 ± 0.64 (321)
27	Bull variation	28,86,88,91,94	NA	21.87 ± 0.42† (310)	–
28 (i)	Season (cold)	95,96	72.67 ± 0.98 (313)	*	*
28(ii)	Season (hot)	95,96	50.75 ± 1.15 (207)	*	*
29 (i)	Follicle size (<3 mm)	52,97,98	40.34 ± 1.25 (166)	*	*
29 (ii)	Follicle size (3–8 mm)	52,97,98	63.08 ± 1.14 (166)	*	*
29 (iii)	Follicle size (>8 mm)	52,97,98	73.07 ± 1.17 (166)	*	*
30	Ovum pick-up	93,99–103	*	64.38 ± 1.84 (433)	20.39 ± 0.45 (387)

Note: Numbers in parentheses are sample size considered for meta-analysis. NA, not applicable; EGF: Epidermal growth factor; IGF: Insulin like growth factor; E2: Estradiol.

* Data not available from at least two articles.

† Fertilization rate.

The pooled estimate was given by

$$\theta_{DL} = \frac{\sum w_i' \theta_i}{\sum w_i'}$$

with standard error

$$SE(\theta_{DL}) = \frac{1}{\sqrt{\sum w_i'}}.$$

In the current study, because no heterogeneity between the studies was observed, all the studies were pooled using the fixed-effect model after statistical analysis. The heterogeneity between the studies was not statistically significant, and τ^2 was zero for all the parameters across the factors considered in the study.

3. Results

The maturation, fertilization/cleavage, and embryo production rates after subjecting the data to meta-analysis for all these factors are presented in Table 1. The maturation rates, cleavage rates, and morulae/blastocyst yield of oocytes matured in medium containing serum versus follicular fluid were 80.56 ± 0.06% versus 75.6 ± 0.16%, 51.79 ± 0.14% versus 40.68 ± 0.21%, and 16.08 ± 0.09% versus 12.61 ± 0.19%, respectively. Similarly, the maturation

rates, cleavage rates, and morulae/blastocyst yield of oocytes matured in medium containing follicle-stimulating hormone plus luteinizing hormone plus estradiol (FSH+LH+E) versus FSH alone versus Pregnant mare serum globulin (PMSG) were 71.73 ± 0.16% versus 77.09 ± 0.23% versus 86.08 ± 0.10%, 53.21 ± 0.14% versus 43.64 ± 0.12% versus 52.74 ± 0.15%, and 18.27 ± 0.10% versus 19.51 ± 0.17% versus 17.16 ± 0.27%, respectively. The corresponding values in oocytes matured in defined media were 82.88 ± 0.48%, 48.57 ± 0.61%, and 15.49 ± 0.42%. The cleavage rates and morulae/blastocyst yield of oocytes recovered by ovum pick-up were 64.38 ± 1.84% and 20.39 ± 0.45%, respectively. The overall maturation, fertilization/cleavage, and embryo production rates in buffalo after subjecting all the data to meta-analysis were 78.36 ± 2.16%, 52.14 ± 1.15%, and 22.14 ± 2.17%, respectively.

4. Discussion

Meta-analysis can be defined as a systematic statistical method for analyzing and synthesizing results from independent studies, taking into account all pertinent information. Traditionally, individuals often seek information from narrative reviews written by experts in the particular field and then use subjective methods to collect and interpret information. Readers of narrative studies may face problems such as lack of detailed description or varied results, and hence the readers may not replicate and verify the results and conclusions of the review. Reviews of a scientific work aim to reduce bias and imprecision and to provide detailed information to allow replication by others. Two of the most effective mechanisms for systematic reviews to reduce bias and imprecision are to include the maximum possible number of relevant individual studies and to provide a detailed description of their strengths and limitations.

The reviews commonly lack a systematic calculation formula to evaluate the various outcomes. The authors use narrative description to illustrate point of interest and deliver it to the reader with detailed information. A review sometimes results in bias and may not give the exact outcomes, as it ignores sample size, effect size, and research design. By synthesizing, scrutinizing, tabulating, and perhaps integrating all relevant studies, meta-analysis allows a more objective appraisal. Thus, meta-analysis is a scientific activity that borrows from both the expert review and the methodology of multicenter studies.

Buffalo oocytes are mostly cultured in groups (5 to 15 oocytes) for 24 h in 50 to 100 μL droplets of complex medium like Tissue Culture Medium (TCM) [13,26,35,37,48,78–81] or Ham's F-10 medium [13,26,35,37,48,78–81] or Minimum Essential Medium (MEM) [35,48,82] under paraffin oil at 38.5 °C in a CO_2 incubator (5% CO_2 and 90% to 95% relative humidity). The basic maturation medium may be supplemented with serum [11–33], hormones like FSH alone [13–16,20,30,32,47–51] or FSH+LH+E [13,31,26,34–46] or FSH+LH [13,19,52–54] or PMSG [11,14,15,39,48,55–59], and in some cases feeder cells [56,60,78]. Follicular fluid was also supplemented with varying result [11,12,23,51,60–65]. Our analysis suggested that TCM-199 may be considered as the best basic medium for buffalo oocytes. Similarly, use of serum instead of follicular fluid in maturation medium resulted in higher maturation and further development to blastocysts. Use of PMSG in maturation medium resulted in comparable and better maturation and subsequent embryonic development compared with that of FSH or other hormones. Cysteamine, which acts as an apoptosis blocker and free radical scavenger, also could improve in vitro oocyte development rates. In contrast, bovine serum albumin (BSA), β-mercaptoethanol, estradiol, or any growth factor supplementations did not further improve the success rates compared with those of conventional protocols.

In vitro–matured oocytes were co-incubated with frozen-thawed in vitro–capacitated spermatozoa in fertilization medium for 24 to 48 h at 38.5 °C in 5% CO_2. Though the current success rate in fertilization rate is over 80%, the cleavage rates remain in the range of 45% to 50%. Considerable variability exists among different bulls in terms of the ability of their spermatozoa to fertilize oocytes in vitro [28,86,88,91,94]. Our analysis indicated that the basic sperm processing and fertilization medium Brackett-Oliphant (BO) resulted in higher cleavage rates. However, the blastocyst production rates were higher in oocytes fertilized in Tyrode's albumin lactate pyruvate (TALP) medium. Both caffeine and theophylline were used as sperm motility enhancer in buffalo IVF. Our analysis suggested that higher cleavage rates in oocytes could be achieved when inseminated with processed sperm supplemented with caffeine rather than theophylline. Buffalo oocytes were inseminated with different concentrations of sperm ranging from 1 million/mL to 10 million/mL. Our analysis indicated that higher concentrations of sperm during oocyte-sperm co-culture resulted in higher cleavage rates.

The weakest aspect of the IVF technology in buffalo is the culture of embryos to transferable stage, as the blastocyst production from cleaved embryos was only

20% to 25%, though our understanding of the requirements for the development of embryos to blastocyst stage has progressed enormously. Our analysis indicated that the development of oocytes recovered from live buffaloes by ovum pick-up techniques was comparable with that of oocytes recovered from slaughterhouse ovaries. This is particularly important for disease control and selection of elite donors. Similarly, the development of oocytes in defined media was comparable with that in complex media. In fact, in vitro embryo production should move toward serum-free defined media for its simple procedures, reduced between-laboratory and within-laboratory variations, and to ensure biosafety [69].

Since the birth of the first buffalo calf from an IVF oocyte [104], a number of articles on in vitro embryo production systems illustrated the effects of different protocols and medium conditions on oocyte and embryo development. Our meta-analysis considered more than 100 articles published from 1991 to 2008 and showed overall values of a high rate of maturation (80%), moderate rate of cleavage (50%), and a moderate to low rate of blastocyst formation (20%) in buffalo. This is in accordance with earlier classic reviews on IVF in buffalo [1,2,105].

There are mixed reports about treatment effectiveness. Some studies may show an effect whereas others do not. Meta-analysis is a set of statistical techniques for combining information from different studies to derive an overall estimate of a treatment's effect [106]. When large, high-quality, randomized and controlled trials are available, systematic review of randomized trials is the gold standard for appraising evidence from trials [107]. The current meta-analysis may represent the gold standard for current status of the in vitro maturation, cleavage, and morulae/blastocyst yield of buffalo oocytes. Our analysis suggests that oocytes with good cumulus layers obtained from large-sized follicles in cold seasons when cultured in TCM-199 supplemented with serum, FSH, and cysteamine resulted in maximum maturation, cleavage, and morulae/blastocysts yield. The results obtained in the current study may be considered for formulation of a simulation model in establishing a cost-effective suitable method for buffalo IVF in further planned research.

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