Asian Pacific Journal of Reproduction

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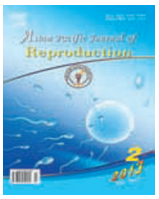

Document heading doi: 10.1016/S2305-0500(13)60129-3

Effect of sera of normal cycling, pregnant and repeat breeding buffaloes (*Bubalus bubalis*) on *in vitro* maturation of buffalo, sheep and goat oocytes

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ARTICLE INFO

Article history:
Received 19 March April 2013
Received in revised form 29 April 2013
Accepted 2 May 2013
Available online 20 June 2013

Keywords:
IVM
Oocytes
Reproductive status
Sera
Buffalo
Sheep
Goat

ABSTRACT

Objective: To examine the oocytes maturation capacity of buffalo, sheep and goat using media containing sera of three different groups of buffaloes (regularly cycling, pregnant and repeat breeding). **Methods:** The buffalo, sheep and goat oocytes were matured under suitable conditions in medium containing sera of regularly cycling, pregnant and repeat breeding buffaloes. **Results:** The oocytes maturation rate containing buffalo oocytes cultured in media containing sera of the control group and regularly cycling group were not significantly different. However when oocytes cultured in the media containing sera of pregnant buffaloes the maturation rate were significantly declined. Further significant declined in maturation rate were observed when oocytes cultured in media containing sera of repeat breeding buffaloes. When sheep and goat oocytes cultured in the media containing control, pregnant and regularly cycling animals sera the oocytes maturation rate were not significantly different. A significant decline in maturation rate of sheep and goat oocytes were observed, when oocytes cultured in media containing sera of repeat breeding buffaloes. **Conclusion:** We may conclude that serum collected from normal cycling buffaloes can be used for oocytes maturation in both homogeneous and heterogeneous species.

1. Introduction

Serum has been used as a source of protein buffering agent and as an agent that protects the oocytes/embryo against the chemical and physical shocks, during culture of oocytes *in vitro* [1]. The maturation rate was found significantly higher in buffalo estrus serum as compared to fetal calf serum and buffalo calf serum [1]. The maturation rates of buffalo oocytes were found to be 51.69%, 47.4% and 53.4 % using buffalo estrus serum [2-4]. In contrast, higher maturation rates viz., 76.6 % and 70.1% were also reported [1,3]. The difference in oocyte maturation rates may be due to the effect of higher number of good quality oocytes obtained during a particular season which are collected over a period of 12 months including adverse summer season making the average of good quality oocytes comparatively lower than obtained by [3] which certainly affects the maturation rates. The difference may also be due to culture conditions [5], or source of serum and the seasons during which the oocytes were collected and matured *in vitro* [6] which have been reported to influence the rate of maturation of oocytes *in vitro*.

The maturation medium with the selection of protein supplements and hormones for *in vitro* maturation plays an important role in subsequent *in vitro* fertilization and *in vitro* development of oocytes [7]. Maturation of the oocytes includes two aspects: nuclear and cytoplasm maturation [8]. *In vitro* maturation of oocytes provide an excellent opportunity for cheap and abundant embryo for carrying out basic

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research and for the application of emerging biotechnologies like cloning and transgenic embryo technology [9]. Though the sera and media from various sources have been used for *in vitro* maturation of buffalo oocytes but the information is still incomplete on this aspect. The present work was undertaken to examine the maturation capacities of buffalo, sheep and goat oocytes using sera of repeat breeding, pregnant and regularly cycling buffaloes.

2. Materials and methods

Ovaries were collected from the civil slaughter house, Bangalore from mature mixed breed water buffaloes, sheep and goat of unknown reproductive status. Sheep/goat ovaries were brought to the laboratory in normal saline (0.9% NaCl) (room temperature, [10] supplemented with gentamicin (50 mg/mL) within 1 hr of slaughter. Buffalo ovaries were brought to the laboratory in warm normal saline (32-35 ℃), [11] supplemented with gentamicin (50 mg/mL) within 2 hrs of slaughter. In the laboratory, the ovaries were washed once again in normal saline.

2.1. Collection of oocytes

Buffalo: Oocytes were aspirated [11] from all visible follicles on the surface of the ovaries with 18 G needle attached to 5 mL sterilized plastic syringe containing aspiration medium. The aspiration medium consisted of TCM-199 and phosphate buffered saline (PBS) mixed with 0.3% BSA at 1:1 ratio. Aspiration of oocytes from all visible surface follicles of ovaries with and without corpus luteum was adopted for buffalo oocytes due to speed of operation, strong ovarian stroma and low yield of acceptable quality oocytes in buffalo [12]. The aspirated oocytes along with the follicular fluid were then placed in 35 mm petridish.

Sheep/goat: Slicing of ovaries [13] was used to retrieve the oocytes. Ovaries were kept in a big round petridish containing TCM-199 supplemented with 0.3% bovine serum albumin (BSA) and then sliced with sterile surgical scalpel blade (No. 21) attached to the BP handle.

2.2. Searching of oocytes

The buffalo, sheep and goat oocytes were searched from the dish under zoom stereomicroscope. All the oocytes were picked up by a pipette and placed in 35 mm culture petridish containing 5 mL of oocyte washing medium (TCM-199 supplemented with 0.3% BSA).

2.3. Grading of oocytes

Oocytes were evaluated by visual morphological appearance under zoom stereomicroscope and graded as per the criteria[14-15].

Buffalo oocytes: Grade A: oocytes with more than 4-5 layers of cumulus cells and homogenous, evenly granular gray ooplasm; Grade B: oocytes not having much compaction, with 2-3 layers of cumulus cells and evenly granular ooplasm; Grade C: oocytes with 1-2 layers of cumulus cells or are partially denuded with irregular dark ooplasm; Grade D: oocytes without cumulus cells and having irregular dark ooplasm and Grade E: oocytes with highly expanded or scattered cumulus cells having irregular dark ooplasm.

Sheep/goat oocytes: Grade A: Oocytes with> 5 layers of cumulus cells; Grade B: oocytes with 3-5 layers of cumulus cells; Grade C: oocytes with 1-3 layers of cumulus cells; Grade D: denuded oocytes and Grade E: oocytes with expanded cumulus cells.

2.4. Selection of oocytes

Only grade A and grade B oocytes (both sheep and buffalo) were used for in vitro culture. Grade C, D and E were considered as poor quality oocytes and hence discarded. Oocytes (>90 mm diameter for ovine oocytes and 144 mm for buffalo oocytes [16] with normal shape were selected for in vitro culture.

2.5. Culture of oocytes for in vitro maturation

The selected oocytes were washed twice in aspiration media and once in the media they would be cultured. The oocytes (6-10 oocytes in a group/droplet) were then transferred into 50 mL droplets of culture media in 35 mm petridish (Tarsons, Kolkata, India). The droplets were covered with paraffin oil and the petridishes were placed in a CO_2 incubator (38.5 ℃, 5% CO_2 in air, 90%-95% relative humidity) for 24 hours.

In control group, oocytes were also cultured in TCM-199 + fetal bovine serum (10%) + FSH-P (5 mg/mL). In the treatment group, the buffalo, sheep and goat oocytes were cultured in a) TCM-199 + serum from repeat breeding buffaloes (10%) + FSH-P (5 mg/mL), b) a) TCM-199 + serum from pregnant buffaloes (10%) + FSH-P (5 mg/mL) and c) a) TCM-199 + serum from regularly cycling buffaloes (10%) + FSH-P (5 mg/mL). All culture media were supplemented with gentamicin (50 mg/mL).

2.6. Evaluation of oocytes for in vitro maturation

The evaluation of cumulus expansion of the oocytes was based on the visual assessment of the degree of expansion (Cumulus expansion Score) under zoom stereomicroscope

as per criteria described by [17]: Degree 0: No expansion; Degree 1 (moderate expansion): Cumulus cells were non-homogenously spread and clustered cells were still observed and Degree 2 (fully expanded): Cumulus cells were homogenously spread and clustered cells were no longer present. Oocytes with moderate and fully expanded cumulus cell masses and unexpanded oocytes with extruded first polar body in the perivitelline space were considered as matured [9].

2.7. Statistical analysis

The data obtained were subjected to statistical analysis using computerized Graph pad prism software. Oocyte maturation capacity values obtained were subjected for one-way ANOVA with tukey's post test.

3. Results

The maturation rate of buffalo, sheep and goat oocytes culture in media containing sera of different reproductive status (repeat breeding, pregnant and regularly cycling animals) of buffaloes were given in Table 1. The maximum maturation rate was observed in buffalo oocytes (84%) when cultured in control group serum of buffalo origin.

Table 1
Effect of sera of different reproductive status of buffaloes on in vitro maturation of buffalo, sheep and goat oocytes.

Serum used	Buffalo oocytes		Sheep oocytes		Goat oocytes	
	Cultured	Matured N (Mean±SEM)	Cultured	Matured N (Mean±SEM)	Cultured	Matured N (Mean±SEM)
Control (FBS)	102	86 (84.1±2.7)^{a}	112	77 (68.6±3.7)^{a}	116	79 (68.0±2.9)^{a}
RBS	100	64 (63.7±2.1)^{c}	102	59 (50.4±4.3)^{c}	102	60 (38.7±1.3)^{c}
PS	108	75 (70.2±2.9)^{b}	104	66 (60.4±1.3)^{b}	106	74 (68.7±3.1)^{b}
RCS	114	88 (77.0±3.0)^{a}	109	66 (63.2±2.7)^{b}	104	71 (68.1±3.9)^{b}

FBS: Fetal bovine serum, RBS: Serum from repeat breeding buffaloes, PS: Serum from pregnant buffaloes, RCS: serum from regularly cycling buffaloes,Values are based on 14 replicates per treatment with 6-10 oocytes per plot, Values with different superscripts in the same column differ significantly.

3.1. Effect of sera of different reproductive status of buffaloes on in vitro maturation of buffaloes oocytes

The oocytes maturation rate containing buffalo oocytes culture in media, the control group and regularly cycling group were not significantly different. However, when oocytes cultured in the media containing sera of pregnant buffaloes the maturation rate were significantly declined. Further, a significant decline in maturation rate was observed when oocytes cultured in media containing sera of repeat breeding buffaloes.

3.2. Effect of sera of different reproductive status of buffaloes on in vitro maturation of sheep oocytes

When sheep oocytes cultured in the media containing control, pregnant and regularly cycling animals sera the oocytes maturation rate were not significantly different. A significant decline in maturation rate of sheep oocytes were observed, when oocytes cultured in media containing sera of repeat breeding buffaloes.

3.3. Effect of sera of different reproductive status of buffaloes on in vitro maturation of goat oocytes

When goat oocytes cultured in the media containing control, pregnant and regularly cycling animals sera the oocytes maturation rate were not significantly different. However, a significant decline in maturation rate of goat oocytes were observed, when oocytes cultured in media containing sera of repeat breeding buffaloes.

4. Discussion

The present study examined the effect of sera of different reproductive status (repeat breeding, pregnant and regularly cycling animals) of buffaloes on *in vitro* maturation of buffalo, goat and sheep oocytes.

Serum has been used as a source of protein buffering agent and as an agent that protects the oocyte/embryo against the chemical and physical shocks, during culture of oocytes *in vitro* [1]. Though the sera and media from various sources have been used for *in vitro* maturation of buffalo oocytes, the maximum maturation rate was observed in buffalo oocytes (84%) when cultured in control group serum of buffalo origin, Our findings are well comparable within range of the findings of other workers [2-4].

The oocytes maturation rate of buffalo oocytes in the culture media, the control group and regularly cycling group were non significantly different however this is according to the findings of other workers [2-4]. When oocytes cultured in the media containing sera of pregnant

buffaloes the maturation rate were significantly decreased further significant decreased in maturation rate were observed when oocytes cultured in media containing sera of repeat breeding buffaloes. The reason may be due to the fact that repeat breeding buffalo sera may contains some oocyte maturation inhibitory factors or some beneficial factors may be present in less quantity.

When sheep oocytes cultured in the media containing control, pregnant and normal cycling buffalo sera the oocytes maturation rate were not significantly different. A significant decrease in maturation rate of sheep oocytes were observed, when oocytes cultured in media containing sera of repeat breeding buffaloes. This may be due to the fact that the repeat breeding buffalo sera may contains some oocyte maturation inhibitory factors or some beneficial factors may be present in less quantity. Similarly, when goat oocytes were cultured in the media containing control, pregnant and regularly cycling buffalo sera, the oocyte maturation rate were not significantly different. However a significant decline in maturation rate of oocytes were observed, when cultured in media containing sera of repeat breeding buffaloes. The reason indicates the decreased beneficial factors or increased oocyte maturation inhibitory factors in sera of repeat breeding buffalo. The normal cycling buffalo serum may provide other physiological active substances such as energy substrates, amino acids, vitamins and hormones which may act in synergy to enhance the maturation of the oocytes. We may conclude that serum collected from normal cycling buffaloes can be used for oocytes maturation in both homogeneous and heterogeneous species.

Conflict of interest statement

We declare that we have no conflict of interest.

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