Improvement of *in vitro* oocyte maturation with lectin supplementation and expression analysis of Cx43, GDF-9, FGF-4 and Fibronectin mRNA transcripts in Buffalo (*Bubalus bubalis*)

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Abstract

Purpose To optimize *In vitro* maturation (IVM) of quality oocytes for embryo production through IVF and SCNT.

Methods Buffalo oocytes were *in vitro* matured in the presence of the pokeweed lectin (*Phytolacca americana*), a potent lymphocyte mitogen. Lectin was supplemented in TCM + 10% FBS at the doses of 0, 1, 5, 10, 15, 20 and 40 µg/ml and cumulus expansion and gene expression patterns were characterized.

Results The degree of cumulus expansion in different lectin treatment levels improved from 1.1 at 1 Ag/ml level to 3.60 at 10 µg/ml level and then decreased in higher concentration 20 µg/ml (1.66) and 40 µg/ml (0.64). IVF embryos showed highest cleavage rate (88.8%) in 10 µg/ml lectin treatment. Expression of all mRNA transcript studied (Cx43, GDF 9, FGF-4 and Fibronectin) was positively correlated with cumulus expansion and polar body extrusion.

Conclusions Mitogenic lectin supplemented maturation media improves oocyte quality for *in vitro* embryo production.

Keywords Oocyte maturation · Lectin · Cx43 · GDF 9 · FGF-4 · Fibronectin

Introduction

Buffalo improvement programme through assisted reproduction is still at research level in laboratories. To improve the IVF and cloned buffalo production the quality IVM

oocytes from slaughter house ovaries needs to supply in abundance. *In vitro* matured oocytes have been commonly used for production of IVF or cloned calves. Complete maturation of oocytes is essential for the developmental competence of embryos [1]. *In vitro* maturation can be accomplished, but is associated with a loss of developmental competence. This loss of developmental competence is associated with the absence of specific proteins in oocytes cultured to metaphase II *in vitro* [2].

The production of embryos in the laboratory involves three major steps: oocyte maturation, fertilization, and embryo culture. Five levels of oocyte developmental competence were characterized by Sirard et al. [3] including the ability to resume meiosis, to cleave upon fertilization, to develop into blastocysts, to induce pregnancy and to generate healthy offspring. Cytoplasmic changes accompanying oocyte growth include mRNA transcription, protein translation and post translational modification [4, 5]. Oocytes are complex cells comprising many organelles and molecules, each of which must be in the appropriate state for the egg to be competent to sustain subsequent embryo development [6]. Although at least 80% of bovine oocytes collected from antral follicles undergo spontaneous nuclear maturation in culture [7], gonadotropins are often added to maturation media to induce cytoplasmic maturation, cumulus expansion and to improve embryonic development. Follicle stimulating hormone (FSH) induces expansion of cumulus oocyte complexes *in vitro* [8]. It improves bovine fertilization and regulates the cleavage rate, growth and reproductive process of the mammalian body [9]. Luteinizing hormone (LH) controls the length and sequence of the female menstrual cycle, including ovulation and ovarian production of both estrogen and progesterone [10]. In addition, it has been reported that serum is required for hormonally induced cumulus

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expansion of COCs, although the percentage may be as low as 0.01–5% [8]. In most cases, supraphysiological hormone concentrations are added to *in vitro* maturation (IVM) media and it is not clear if these high concentrations are strictly required [11]. Recently, recombinant gonadotropins have become commercially available; these are very pure sources of hormone that can allow the individual roles of FSH and LH to be investigated without the hormone cross-contamination of pituitary, serum or urinary preparations.

General mitogens specifically lectins can stimulate maturation of the isolated mouse cumulus oocyte complexes (COCs) [12]. Lectins are carbohydrate-binding proteins or glycoproteins of nonimmune origin that have been shown to have profound effects on a variety of cultured cells including agglutination and mitogenesis [13]. Lectins serve many different biological functions in animals, from the regulation of cell adhesion to glycoprotein synthesis and the control of protein levels in the blood.

The present study was performed to analyze the effects of 0, 1, 5, 10, 20 and 40 µg/ml lectin in maturation media on oocytes maturation. The oocyte maturation was assessed by cumulus cells expansion through cumulus expansion index (CEI), polar body formation and mRNA transcript level of genes related to cell communications and gap junction protein; Cx43 [14]; cumulus-expansion enabling factor and cell cycle protein; GDF-9 [15]; basic growth factor; FGF-4 [16] and cell membrane protein Fibronectin [17].

Materials and methods

All the chemicals used in the present study were purchased from Sigma aldrich company, St. Louis, MO, USA unless otherwise stated. All the experiment was conducted in triplicate.

Collection of cumulus oocyte complexes

Buffalo ovaries were obtained from a nearby abattoir and transported in physiological saline (0.9% NaCl) at 25–30°C to the laboratory within 3 hours after slaughter and washed three times in the physiological saline. Ovarian antral follicles (2–8 mm) were aspirated using 18-gauge needle and collected into Hepes buffered tissue culture medium-199 (TCM-199) plus phosphate buffer saline (Gibco BRL/Life Technologies: Grand Island, NY, USA) supplemented with 0.1% bovine serum albumin (BSA). Cumulus oocyte complexes (COCs) showing an even cytoplasm and surrounded by at least three layers of compact cumulus cells were selected. COCs were washed three times in TCM-199 medium supplemented with 10% fetal bovine serum (FBS; Hyclone: Logan, Utah, USA), gentamycin sulfate (10 µg/ml) and sodium pyruvate (0.2 mM). The oocytes were cultured in group of 20–25 in

0.5 ml TCM-199 medium supplemented with gentamycin sulphate (10 µg/ml), β-estradiol (1,000 IU/ml), FSH (500 IU/ml), LH (500 IU/ml) and 10% FBS in 4-well plates (NUNC, Denmark) at 38.5°C in 5% CO₂ in air, for 24 h. Oocytes kept for maturation were divided in 4 treatment groups with lectin (Cat# L-8777; collected from *Phytolacca americana*) concentration of 0, 1, 5, 10, 20 and 40 µg/ml maturation medium.

Cumulus expansion assessment

Cumulus expansion was scored according to the 0–4 scale and the cumulus expansion index (CEI) was calculated as described by Fagbohun and Downs [18]. Using this scale, score 0 indicates no expansion, characterized by the detachment of cumulus cells from the oocyte to assume a flattened monolayer of fibroblastic appearance leaving a partially or fully denuded oocyte. A score of 1 indicates no expansion but cumulus cells are spherical, and remain compacted around the oocyte. For score 2 complexes, only the outer most layers of cumulus cells have expanded, score 3 complexes have all layers except the corona radiata prominently expanded and a score of 4 indicates the maximum degree of expansion including the corona radiata (cells most proximal to the oocyte) [19].

In vitro fertilization

In vitro matured COCs were fertilized *in vitro* with prepared sperm as per previously described by Madan et al. [20]. One straw of frozen buffalo semen was thawed at 37°C and washed twice by centrifugation at 500×g for 10 min in Brackett and Oliphant [21] medium (BO). After washing, the media was supplemented with 10 mM caffeine, 10 µg/ml Heparin and 0.2 mM sodium pyruvate. The pellet was resuspended in caffeine-BO medium at the concentration of 2×10⁶ sperm/ml. A 50 µl aliquot of semen suspension was added to each 50 µl droplet containing oocytes, and they were co-cultured together for 6 h in CO₂ incubator at 38.5°C. The IVF embryos were cultured at 38.5°C in Hepes buffered TCM-199 supplemented with 10% FBS medium.

Preparation of lysates of cumulus oocyte complexes

Three freshly matured COCs from each lectin treatment were denuded with hyaluronidase enzyme. Oocytes were treated using cells to cDNA II kit (Ambion: Austin, TX, USA) and washed three times with the PBS by centrifugation for 5 min at 4°C at 1,200 g in 0.2 ml centrifuge tubes. Total 100 µl of the cell lysis buffer was added to the pelleted oocytes. To lyse the oocytes, the suspension was heated using a block-type thermal cycler system according to manufacturer's instructions.

DNase digestion and reverse transcription

Oocyte lysates were supplemented with 0.2 μ l (0.4 U/ μ l) of DNase I, and incubated at 37°C for 15 min. After incubation; each tube was heated for 10 min at 75°C to inactivate the DNase immediately. To synthesize the first strand of cDNA, 5 μ l cell lysate, 4 μ l dNTP mix, 2 μ l oligo dT and 5 μ l RNase free water were assembled in a RNase free 0.2 ml tube, then heated for 3 min at 70°C. After cooling the mixture on ice, 2 μ l 10X RT buffer, 1 μ l M-MLV reverse transcriptase and 1 μ l RNase inhibitor were added to the reaction tubes. Reverse transcription was carried out for 1 h at 42°C, followed by incubation at 95°C for 10 min. RT minus product with all the reaction components except of the reverse transcriptase were produced for each sample, and were then employed for real-time PCR in order to demonstrate that the template for the PCR product was cDNA, not genomic DNA.

Primer design

Real time PCR primers for Cx43, GDF-9, FGF-4, Fibronectin, GAPDH and β -actin were designed using the software Beacon Designer 7.0 (Premier Biosoft International, Palo Alto, CA, USA). The primers were carefully designed to avoid amplification of genomic DNA. The sequence of the primers used, the fragment size, annealing temperature and the sequence references of the expected PCR products are shown in Table 1.

Real-time PCR analysis of gene expression

Before the final step of gene expression analysis, each cDNA sample was first amplified with a pair of primers specific for β -actin mRNA in order to screen the samples

for contamination with genomic DNA. Real-time PCR (Mx3000p Stratagene: La Jolla, CA, USA) was performed using the SYBR Green qPCR supermix (Invitrogen SYBR Green qPCR supermix: Carlsbad, CA, USA) as a double-stranded DNA-specific fluorescent dye in 25 μ l reaction to assess the gene expression of Cx43, GDF-9, FGF4 and Fibronectin relative to housekeeping gene GAPDH. All gene of interest were analyzed in three replicates with different samples. To confirm the results each of three replicates was analyzed for quantitative assessment of RNA amplification with PCR primers.

Final concentration 10 nM of forward and reverse primers and cDNA (4 μ l) with 12.5 μ l 2X PCR SYBR Green master mix was added per reaction. Samples not exposed to reverse transcriptase (RT) were used as negative controls. The PCR conditions were 95°C for 10 min, then 50 cycles consisting of denaturation at 95°C for 10 s, then annealing at 56°C for 10 s and extension at 72°C for 15 s. Subsequent to the PCR, a dissociation curve analysis programme was run to confirm a single specific peak and to detect primer/dimer formation using the programme of 0 s at 95°C, 10 s at 56°C then for 0 s at 95°C with acquisition on the step mode. Product identity was confirmed by ethidium bromide-stained 2% gel electrophoresis.

The comparative C_T method was used for relative quantification of target gene expression levels [22]. Quantification was normalized to the internal control GAPDH gene. Within log-linear phase region of the amplification curve, each cycle doubled the amplified product. The ΔC_T value was determined by subtracting the GAPDH C_T value for each sample from the target gene C_T value. Calculation of $\Delta\Delta C_T$ involved using the highest sample method ΔC_T as an arbitrary constant to subtract from all other ΔC_T sample values. Fold changes in relative mRNA expression

Table 1 Details of primers used for real time PCR experiments

Genes and sequence reference (GenBank accession no.)	Primer sequence	Size of PCR product (bp)	Annealing temp
GAPDH (NM_001034034)	5'-TCTGTTGTGGATCTGACCTG-3' 5'-CCAGCATCGAAGGTAGAAGAG-3'	172	55°C
β -Actin (NM_001101)	5' CACGAACTACCTTCAACTC 3' 5' TCTTGATCTTCATTGTGCTG 3'	162	56°C
Cx43 (J05535)	5'-GGAGACAGAAACAATTCTTCC-3' 5'-TTTAGAATTCTGGTGGTCGT-3'	164	55°C
GDF-9 (EF202171)	5'GACAAGTGGAGAAGCTG 3' 5' ARGATGTTGTTGACCATGCT3'	156	56°C
FGF-4 (NM_010202)	5'AGGCGTGGTGAGCATCTTC3' CGTAGGCGTTGTAGTTGTTGG	140	56°C
Fibronectin (NM153388)	5'TGGGAGAACAGTGGCAGAAG3' 5'GTAGGAGTGGGCAGTGGAG3'	144	56°C

of the target genes were determined using the formula $2^{-\Delta\Delta CT}$.

Statistical analysis

Average and standard deviation were calculated for cumulus expansion and polar body formation rate using SPSS statistical programme (SPSS 7.5 for windows; SPSS, Inc., Chicago, IL, USA). The data were considered for significant differences by one way ANOVA using SPSS programme ($P \leq 0.05$).

Relative quantification of target gene expression levels as fold-difference was based on three sets of samples. Data on mRNA expression were analyzed using the light cycler software (Stratagene). After testing for normality and equal variance, one-way ANOVA followed by post-hoc multiple pair wise comparisons using LSD and Duncan's multiple range tests for variable were employed to determine the difference in the gene expression pattern in matured oocytes cultured with different dosage of lectin using the SPSS 7.5 programme. Differences were considered to be significant at $P \leq 0.05$.

Results

Effects of lectin on meiotic progression of COCs

The aim of these experiments was to determine whether lectin supplementation affects nuclear maturation in oocytes. COCs morphology on the basis of compactness provides information about the oocyte maturation quality. The cumulus expansion rate after 24 h of maturation in 0, 1, 5, 10, 20 and 40 $\mu\text{g/ml}$ lectin supplemented maturation medium was 51.5%, 56.9%, 66.1%, 98.4%, 72.3% and 36.9% (Table 2).

The proportion of oocytes with extruded first PB was highest ($P \leq 0.05$) in 10 $\mu\text{g/ml}$ lectin treatment (93.7%) as compare to 0 $\mu\text{g/ml}$ (59.7%), 1 $\mu\text{g/ml}$ (64.4%), 5 $\mu\text{g/ml}$ (71.9%), 20 $\mu\text{g/ml}$ (68.0%) and 40 $\mu\text{g/ml}$ (50.0%) (Table 2).

A comparative study of lectin concentrations on cumulus expansion

Cumulus expansion index (CEI) was also formulated for each treatment group. This involved calculating the average expansion value for a particular lectin treatment. CEI was 0.92, 1.1, 1.89, 3.66, 1.66 and 0.64 at 0, 1, 5, 10, 20 and 40 $\mu\text{g/ml}$ lectin concentrations, respectively (Table 3).

Effect of lectin concentration on embryonic development

Cleavage percentage in medium supplemented with 10 $\mu\text{g/ml}$ lectin concentration was significantly highest (88.8%) compare to 0 $\mu\text{g/ml}$ (60.2%), 1 $\mu\text{g/ml}$ (64.4%), 5 $\mu\text{g/ml}$ (74.6%), 10 $\mu\text{g/ml}$ (88.8%), 20 $\mu\text{g/ml}$ (62.2%) and 40 $\mu\text{g/ml}$ (12.4%) lectin dosage (Table 4).

Effects of lectin on levels of specific transcripts in oocytes

The mRNA levels of Cx43, GDF-9, FGF-4 and Fibronectin were determined by Real time PCR, to evaluate the effect of lectin supplementation in matured oocytes. The mRNA expression of all the four genes were increased with amount of lectin supplementation in media up to 10 $\mu\text{g/ml}$ level but decline thereafter at 20 $\mu\text{g/ml}$ to 40 $\mu\text{g/ml}$ dosage. There was no significant effect of 1 and 40 $\mu\text{g/ml}$ lectin dose on Cx43 (Fig. 1) and GDF-9 (Fig. 2) expressions in matured oocytes, whereas FGF-4 (Fig. 3) and Fibronectin (Fig. 4) mRNA abundance in matured oocytes was also increased ($P \leq 0.05$) by the lower dosage (1 $\mu\text{g/ml}$ and 5 $\mu\text{g/ml}$) as compare to control (0 $\mu\text{g/ml}$).

Overall, cumulus expansion and polar body formation rates were higher in 10 $\mu\text{g/ml}$ lectin treatment. Lectin at higher concentration 20 $\mu\text{g/ml}$ and 40 $\mu\text{g/ml}$ was detrimental to oocyte maturation. At these concentrations the cumulus expansion and polar body formation rate decreased significantly. Around 32% and 50% expanded oocytes in 20 and 40 $\mu\text{g/ml}$ lectin concentrations did not form polar bodies. Cleavage percentage of IVF embryos produced from 10 $\mu\text{g/ml}$ lectin concentration was significantly higher in comparison to

Table 2 Oocyte developmental rate in different concentration of lectins

Groups	Oocytes cultured	Cumulus expansion	Cumulus expansion rate (%)	Polar body	Polar body formation rate (%)
0 $\mu\text{g/ml}$ lectin	130	67	51.1 $\pm$ 2.5	40	59.7 $\pm$ 3.2
1 $\mu\text{g/ml}$ lectin	130	76	56.9 $\pm$ 1.8	49	64.4 $\pm$ 2.0
5 $\mu\text{g/ml}$ lectin	130	89	66.1 $\pm$ 2.4	64	71.9 $\pm$ 1.4
10 $\mu\text{g/ml}$ lectin	130	128	98.4 $\pm$ 2.0	118	93.7 $\pm$ 3.4
20 $\mu\text{g/ml}$ lectin	130	94	72.3 $\pm$ 3.5	64	68.0 $\pm$ 1.8
40 $\mu\text{g/ml}$ lectin	130	48	36.9 $\pm$ 3.2	24	50.0 $\pm$ 1.2

Table 3 Effects of lectin dosage on cumulus cell expansion

Treatment	Degree of cumulus expansion						
	n	0	+1	+2	+3	+4	CEI
0 $\mu\text{g/ml}$	130	63	32	20	12	3	0.92
1 $\mu\text{g/ml}$	130	54	33	23	16	4	1.1
5 $\mu\text{g/ml}$	130	41	12	25	24	28	1.89
10 $\mu\text{g/ml}$	130	1	2	4	25	98	3.66
20 $\mu\text{g/ml}$	130	36	30	20	30	74	1.66
40 $\mu\text{g/ml}$	130	82	20	20	8	0	0.64

its other counterparts. Relative abundance of all the four genes were increased ($P \leq 0.05$) in 10 $\mu\text{g/ml}$ lectin compared to other treatment levels.

Discussion

The developmental potential of oocytes matured *in vitro* is reduced compared to oocytes matured *in vivo* [23]. In order to enhance the bubaline oocyte maturation rate, it is necessary to modify a suitable oocyte maturation medium with different supplements. The mitogenicity of lectins is strongly associated with the stimulation of germinal vesicle breakdown (GVB) as well as cumulus expansion. Fagbohun and Down [18] also reported that the dosage of lectin concentration and maturation rate of the COCs are directly proportional to each other up to some level. The present study demonstrates that lectin doses enhance both oocyte maturation and regulation of gene expression in oocytes.

The processes of meiotic maturation and acquisition of developmental competence determine the ability of the oocyte to undergo successful fertilization, cleavage, and embryonic development. These important steps are dependent upon a variety of factors that lead to proper nuclear and cytoplasmic maturation [24]. Oocyte meiotic maturation is a complex process that involves germinal vesicle breakdown, chromosome condensation and segregation,

Table 4 Embryo production rate

Groups	Cleavage (%)
0 $\mu\text{g/ml}$ lectin	60.2 $\pm$ 1.6
1 $\mu\text{g/ml}$ lectin	64.4 $\pm$ 1.8
5 $\mu\text{g/ml}$ lectin	74.6 $\pm$ 3.2
10 $\mu\text{g/ml}$ lectin	88.8 $\pm$ 1.1
20 $\mu\text{g/ml}$ lectin	62.2 $\pm$ 2.2
40 $\mu\text{g/ml}$ lectin	12.4 $\pm$ 1.2

Fig. 1 Relative transcript expression level of Cx43 gene in matured oocytes cultured in IVM medium supplemented with 0, 1, 5, 10, 20 and 40 $\mu\text{g/ml}$ lectin concentration. Bars with different superscript within a panel differ significantly ($P \leq 0.05$). The standard deviation is indicated by error bars. The vertical axis indicates the relative mRNA abundance, and the horizontal axis indicates the lectin concentrations

formation of the metaphase plate, completion of meiosis I, extrusion of the first PB, and arrest at metaphase II [25].

It is well known that oocyte developmental potential is a reflection of proper cytoplasmic maturation. Even though most bovine oocytes resume meiosis and progress to metaphase II following IVM [26], cytoplasmic *in vitro* maturation is generally compromised, leading to low rates of development. In the *in vitro* culture system of human COCs, the number of cumulus cells in COCs before culture was positively correlated ($P \leq 0.05$) with the developmental competence of oocytes after *in vitro* maturation [27], suggesting that a sufficient number of cumulus cells is required to support oocyte maturation. To mimic the changes observed in *in vivo* oocytes, immature COCs from early stage follicles were cultured with a specific temporal sequence of FSH, LH, β -estradiol and hCG along with

Fig. 2 Relative transcript expression level of GDF9 gene in matured oocytes cultured in IVM medium supplemented with 0, 1, 5, 10, 20 and 40 $\mu\text{g/ml}$ lectin concentration. Bars with different superscript within a panel differ significantly ($P \leq 0.05$). The standard deviation is indicated by error bars. The vertical axis indicates the relative mRNA abundance, and the horizontal axis indicates the lectin concentrations

Fig. 3 Relative transcript expression level of FGF4 gene in matured oocytes cultured in IVM medium supplemented with 0, 1, 5, 10, 20 and 40 µg/ml lectin concentration. Bars with different superscript within a panel differ significantly ($P \leq 0.05$). The standard deviation is indicated by error bars. The vertical axis indicates the relative mRNA abundance, and the horizontal axis indicates the lectin concentrations

different concentration of lectin (0 µg/ml, 1 µg/ml, 5 µg/ml, 10 µg/ml, 20 µg/ml and 40 µg/ml). Pokeweed lectin (PL), a lectin for N-acetylglucosamine containing saccharides, stimulating peripheral lymphocytes to undergo mitosis in cultures. The addition of lectin during the maturation of bovine COCs increased cumulus expansion, polar body (PB) extrusion and the proportion of oocytes that reached the metaphase II stage [18]. Our finding also support that lectin acts in cumulus-enclosed oocytes to stimulate cumulus expansion rate up to 98.4% and polar body formation rate 93.7%.

The 10 µg/ml lectin concentrations was optimum and showed a significant effect on oocyte maturation and further development. At high concentrations of lectin (20 µg/ml and 40 µg/ml), we observed decrease in cumulus

Fig. 4 Relative transcript expression level of Fibronectin gene in matured oocytes cultured in IVM medium supplemented with 0, 1, 5, 10, 20 and 40 µg/ml lectin concentration. Bars with different superscript within a panel differ significantly ($P \leq 0.05$). The standard deviation is indicated by error bars. The vertical axis indicates the relative mRNA abundance, and the horizontal axis indicates the lectin concentrations

expansion rate, polar body formation rate and CEI. It demonstrates that higher dosage of lectin partially inhibit maturation process, whereas, low dosage of lectin (1 µg/ml and 5 µg/ml) due to the binding of culture medium component to lectin, caused inhibition of stimulated effect on oocyte maturation and further development. Oocytes matured in each lectin treatment groups were subjected to *in vitro* fertilization. The cleavage of IVF oocytes in 10 µg/ml lectin concentration was significantly higher compare to other treatment groups as well as control. These results showed that lectin treatment did not interfere with the IVF procedure.

Lectins may influence the synthesis and release of cumulus cell-derived factors, which reach the oocyte through gap junction coupling and the extracellular environment. Cx43 mRNA was expressed in all the lectin treatments and control (0 µg/ml) group. Cx43 mRNA abundance was increased up to 10 µg/ml and then declined in 20 µg/ml to 40 µg/ml lectin treatment groups.

Lectins stimulate the mitogen activated protein (MAP) kinase pathway, subsequently leading to oocyte meiotic maturation, similarly recombinant GDF9 has also been shown to activate the MAP kinase pathway during oocyte meiotic maturation [28]. Lectin caused a greater increase in the GDF-9 transcript level also in matured oocytes treated with 5 µg/ml and 10 µg/ml lectin dosage. The expression pattern was similar to that of Cx43, and showed decrease in 20 µg/ml and 40 µg/ml lectin treatment groups.

Lectin up regulated the transcript levels ($P \leq 0.05$) of the growth factor (FGF-4) and cell membrane protein (Fibronectin) up to 10 µg/ml dose level. FGF-4 gene is implicated in the cytoplasmic activity and cell mediated signaling pathways. FGF-4 are heparin-binding proteins and interactions with cell-surface associated heparan sulfate have been shown to be essential for FGF signal transduction [29]. FGF-4 is key-players in the processes of proliferation and differentiation of oocytes. Fibronectin is a multifunctional protein plays an important role in integrin-mediated cell adhesion, composition and assembly of extracellular matrix, and multi-modular protein structure [30].

In conclusion, lectin acts directly in the oocyte to enhance meiotic maturation and development potential. The genes encodes for oocyte maturation products are required for early embryonic development. Lectin-induced maturation and differential regulation of genes involved in different physiological activities like gap junction and cell communication protein (Cx43), cumulus-expansion enabling factor and cell cycle protein (GDF-9), basic growth factor (FGF-4) and cell membrane protein (Fibronectin) can be associated with the developmental competence vis a vis improvement in IVM rate which will further improve the IVF and SCNT buffalo calves production in India.

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