

Vitrification and Subsequent *In Vitro* Maturation of Mouse Preantral Follicles in Presence of Growth Factors

Zahra Oryan Abkenar, M.Sc.¹, Roya Ganji, M.Sc.², Amir Eghbal Khajehrahimi, Ph.D.³,
Mohammad Hadi Bahadori, Ph.D.^{4*}

1. Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Iran

2. Faculty of Biology, Tarbiat Moallem University, Tehran, Iran

3. Branch of North of Tehran, Islamic Azad University, Tehran, Iran

4. Cellular and Molecular Research Center, Faculty of Medicine, Guilan University of Medical Science, Rasht, Iran

*Corresponding Address: Cellular and Molecular Research Center, Faculty of Medicine, Guilan University of Medical Science, Rasht, Iran
Email: Bahadori.mh@gmail.com

Received: 16/Feb/2013, Accepted: 21/Oct/2013

Abstract

Objective: Cryopreservation of ovarian tissue or follicles has been proposed as an alternative method for fertility preservation. Although successful vitrification of follicles has been reported in several mammalian species, the survival rate is generally low. The aim of this study was to investigate the effects of fibroblast growth factor (FGF) and epidermal growth factor (EGF) on *in vitro* preantral follicle development after vitrification.

Materials and Methods: In this experimental study, preantral follicles with diameter of 150-180 μm were mechanically isolated from ovaries of 18-21 days old NMRI mice. Follicles were vitrified and warmed, then cultured in α -minimal essential medium (α -MEM) without growth factor supplementation as control group (group I), while supplemented with 20 ng/ml FGF (group II), 20 ng/ml EGF (group III), and 20 ng/ml FGF +20 ng/ml EGF (group IV). After 12 days, human chorionic gonadotrophin (hCG)/EGF was added to culture medium, and after 18-20 hours, the presence of cumulus oocyte complexes (COCs) and oocyte maturation were assessed. The chi-square (χ^2) test was used to analyze survival and ovulation rates of the follicles.

Results: Our results showed that the rate of metaphase II (MII) oocytes in FGF group increased in comparison with control and other treatment groups ($p < 0.027$), but there was no difference between control with EGF and EGF+FGF groups in oocyte maturation rate ($p > 0.05$). There was a significant decrease in survival rate of follicles in EGF+EGF group in comparison with other groups ($p < 0.008$). After *in vitro* ovulation induction, the follicles in EGF group showed a higher ovulation rate ($p < 0.008$) than those cultured in other groups.

Conclusion: FGF has beneficial effect on oocyte maturation, and EGF increases COCs number *in vitro*. Combination of EGF and FGF decreases the number of survived follicles.

Keywords: Vitrification, Mouse Preantral Follicle, *In Vitro* Maturation, Epidermal Growth Factor, Fibroblast Growth Factor

Cell Journal (Yakhteh), Vol 16, No 3, Autumn 2014, Pages: 271-278

Citation: Oryan Abkenar Z, Ganji R, Eghbal Khajehrahimi A, Bahadori MH. Vitrification and subsequent *in vitro* maturation of mouse Preantral follicles in presence of growth factors. Cell J. 2014; 16(3): 271-278.

Introduction

Cryopreservation of ovarian tissue or follicles has been proposed as an alternative method for fertility preservation (1-3). After isolation of follicles from ovarian tissue using enzymatic or mechanical techniques, follicles would require further *in vitro* maturation (4-6). The penetration of the cryoprotectant agent into the follicular structure is easier compared to ovarian tissue (4). In addition, the assessment of follicles after thawing is easier than whole ovarian tissue (7). For these reasons, cryopreservation of isolated ovarian follicles seems to be more attractive option than other methods (8). Although vitrification is considered as a simple cryopreservation method, the requirement steps for high concentrations of cryoprotectant cause osmotic and toxic damage to cells (9).

It is demonstrated that culture of follicles isolated from frozen/thawed ovarian tissue produces mature oocytes, but the diameter of these follicles is smaller than that of fresh ones (10). The primordial follicle is the earliest form of follicle in the ovaries that initiates the next phase of development under different unknown signals (11). Only a few follicles reach to the ovulation stage. The mechanism by which the primordial follicles develop to preantral stage is yet unclear.

The immature oocytes are not injured during the process of vitrification due to following factors: small size, few developments, few organelles, absence of zona pellucid and low metabolism. Culture of ovarian follicles is an alternative method for fertility treatment. Recently various culture systems for preantral follicles and oocyte-granulosa cell complexes have also been proposed (12-13).

The factors and mechanisms involved in this process are not yet fully understood. Irrespective of gonadotropin involvement, there is good evidence suggesting that local regulatory factors are implicated in this temporal and spatial process (14, 15).

The fibroblast growth factors (FGF-4s), as heparin-binding single chain polypeptides, have a crucial role in development, cell growth and tissue repair.

They stimulate the ovarian granulosa cell differentiation (16), the expression of the luteinizing hormone (LH) receptors by granulosa cells, and the proliferation of ovarian germinal cells (17). Epidermal growth factor (EGF) results in cellular proliferation and survival (18). Furthermore, EGF plays a role in oocyte *in vitro* maturation (19),

while stimulates the proliferation of granulosa cells *in vivo* and *in vitro* (20). In human oocytes, the expressions of EGF and its receptor is detected in follicles at preantral stage (21).

The aim of this study was to investigate the effects of fibroblast growth factor (FGF) and epidermal growth factor (EGF) on *in vitro* preantral follicle development after vitrification.

Materials and Methods

Animals

In this experimental study, all female mice used in this study were obtained from the Razi Institute, Karaj, Iran. The animals were housed individually in an air-conditioned controlled room at 23-25°C and under a 12 hour light: 12 hours dark cycle (6 am: 6 pm), fed a commercial diet, and given water ad libitum. All the animal experimentations were approved by the Animal Ethics Committee at the Guilan University of Medical Sciences (GUMS).

Isolation of preantral follicles

Ovaries of prepubertal Naval Medical Research Institute mice (aged 18-21 days) were aseptically removed from the animals after being killed and placed in rewarmed isolation medium, consisting of alpha-minimum essential medium (α -MEM; Gibco-Invitrogen, Germany) supplemented with 10% v/v fetal bovine serum (FBS, Sigma, Germany) and 100 IU/ml penicillin +100 μ g/ml streptomycin (Sigma, Germany). The ovaries were mechanically dissected using fine hypodermic needles (a 26-gauge). Follicles with a diameter in the range 150-180 μ m were then collected.

Vitrification procedure

Preantral follicles were vitrified using an ethylene glycol (EG) and dimethyl sulfoxide (DMSO, Sigma, Germany) based on the protocol. The base media for the preparation of equilibration and vitrification solutions was α -MEM + 20% FBS. Follicles were equilibrated for 3 minutes in 7.5% equilibration solution containing 7.5% EG + 7.5% DMSO followed by a 30-40 second incubation in vitrification solution containing 15% EG + 15% DMSO + 0.5M sucrose. As soon as cellular shrinkage was observed, five preantral follicles were aspirated and placed on the tip of the cryolock (Biodiseno, USA). Cooling of the preantral follicles was done by direct contact with liquid nitrogen. The cryolocks were stored in liquid nitrogen for

30 days. All vitrification procedures were performed at room temperature.

Warming

For warming, the cryolocks containing vitrified preantral follicles were held in air for 20 seconds at room temperature. Then they were exposed with warming solutions by serial dilution in base medium with decreasing concentration of sucrose from 1 M to 0.5 M and 0.5 M to 0 M for 1-3 and 40-45 minutes, respectively. All procedures were carried out at room temperature, but the last step in base medium was performed at 37°C.

Evaluation of immediate post-warming survival rate

Survival of vitrified/warmed follicles was assessed microscopically based on morphology of the follicle under a stereomicroscope followed by inverted microscope (IX71, Olympus, Japan). A follicle was considered to be intact if it possessed an oocyte surrounded by a complete tight collar of granulosa cells (GCs). Follicles with partially or completely naked oocytes or large spaces within the granulosa-oocyte complex were graded as damaged. Any dark artistic-looking follicles were also graded as damaged. Only intact preantral follicles were selected for further *in vitro* culture (IVC).

In vitro culture of vitrified-warmed preantral follicles

Vitrified/warmed preantral follicles were individually cultured in 20 µl droplets of maturation medium containing α -MEM supplemented with 1% insulin, transferrin, and selenium ITS (Invitrogen, USA); 100 mIU/mL recombinant human follicle stimulating hormone (rhFSH, (Gonal-f, Merck Serono, Switzerland), and 5% FBS (Sigma, Germany). The follicles were cultured for 12 days at 37°C in an atmosphere of 5% CO₂ in 60×15 mm Petri dish (Falcon, USA) covered with 5ml mineral oil (Sigma, Germany).

Experimental groups

Vitrified/warmed preantral follicles were cultured in maturation medium without growth factor supplementation as control group (group I), while supplemented with 20 ng/ml FGF (group II), 20 ng/ml EGF (group III) and with combination of 20 ng/ml EGF and 20 ng/ml FGF (group IV).

Ovulation induction

On day 12 of culture, 1.5 IU/ml recombinant hu-

man chorionic gonadotrophin (rhCG) and 5 ng/ml recombinant epidermal growth factor (rEGF) were added to the cultures as *in vitro* ovulatory stimulus. Optimal nuclear maturation rate was achieved after 18-20 hours of induction (22).

Statistical analysis

We used the chi-square (χ^2) test to analyze survival and ovulation rates of the follicles and the nuclear maturation of the oocytes. Data analysis was performed using Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago, IL, USA) version 16.

Results

Survival of vitrified-warmed preantral follicles

On day 1 of culture, the healthy follicles had attached to the culture dish (Fig 1A).

On day 12, there was a significant ($p < 0.008$) decrease in the number of survived follicles (67.8%) in group III (FGF+EGF) in comparison with control group. There was no significant difference ($p > 0.05$) between control, FGF and EGF groups, while their survival rates were 84.6, 79.3 and 77.3%, respectively.

In vitro ovulation of vitrified-warmed preantral follicles

At the end of the culture (Fig 1B), the follicles were stimulated by rhCG and rEGF to induce ovulation. Cumulus-oocyte complexes (COCs) were expanded and released after 18-20 hours of stimulating follicles (Fig 1C).

Our results revealed significant differences ($p < 0.05$) in the proportion of ovulated COCs between the control and EGF groups. The highest percentage of released COCs (31.4%) was observed in group III with 20 ng/ml EGF as compared to other groups (13.6, 14.5, and 12.5% in control, II and IV groups, respectively) ($p < 0.008$) (Fig 2).

Maturation state of oocytes

The percentage rates of oocytes reaching to MII stage (Fig 1D) were 39.5, 66.7, 61.5, and 50% in the control, II, III and IV groups, respectively. We observed significant maturation rate in 20 ng/ml of FGF (66.7%, $p < 0.027$) compared with those cultured in the other groups (Fig 2).

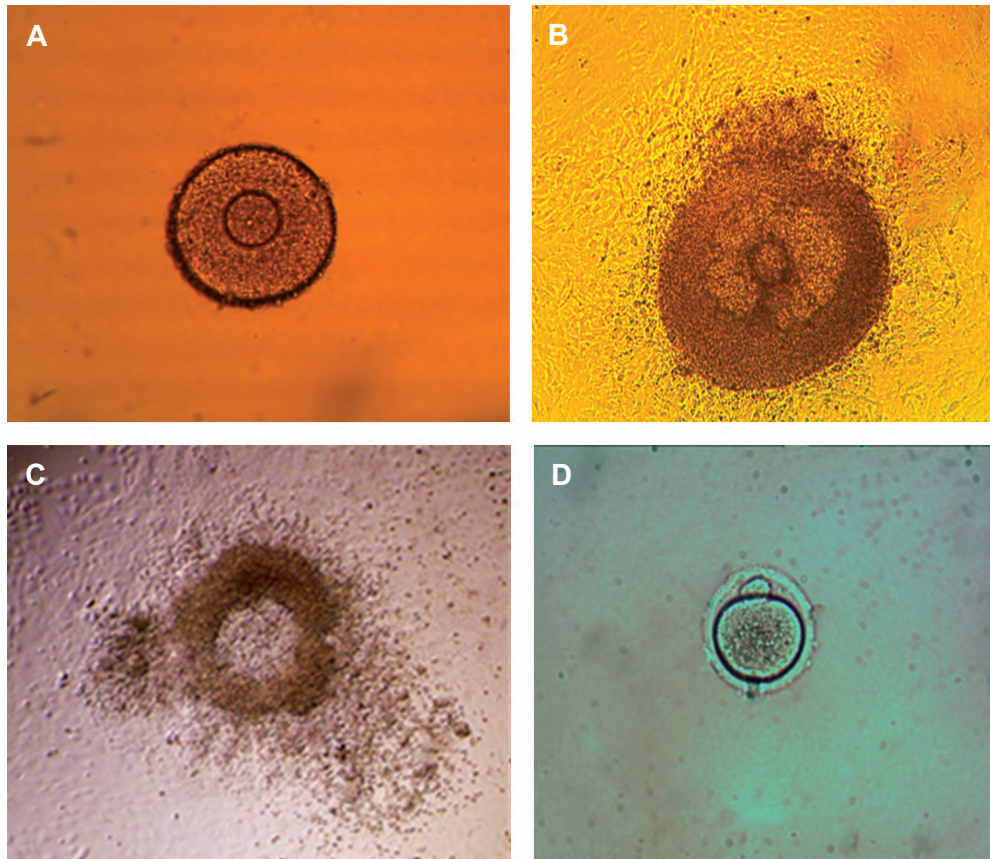


Fig 1: Preantral follicle on day 1 of culture (EGF group) ($\times 100$) (A), an antral follicle after 10 days of culture (EGF group) ($\times 100$) (B), an antral follicle stimulated with hCG/EGF showing COC extraction (EGF group) ($\times 40$) (C) and MII oocyte after in vitro ovulation (EGF group) ($\times 400$) (D).

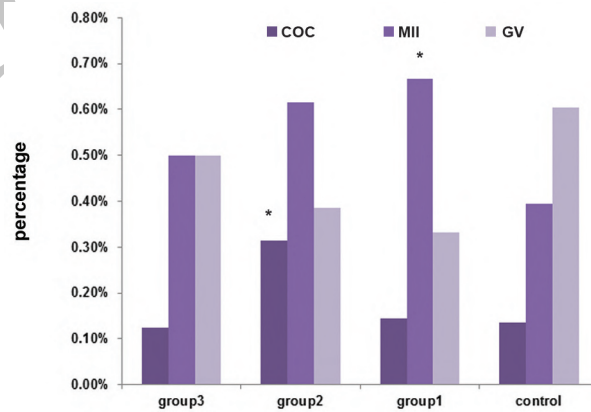


Fig 2: Oocyte maturation and in vitro ovulation. High maturation rate was observed in FGF group and high ovulation rate was observed in EGF group. Statistical differences are indicated above the columns and *; $P < 0.05$.

Discussion

Freezing and thawing procedures can decrease the growth rate of oocytes and granulosa cells in the follicles.

Cryopreservation depends on survival rates of the granulosa cells and oocytes and on the maintenance of gap junctions between the granulosa cells and oocytes (23). Recent studies have indicated high rate of follicular survival with normal morphology after cryopreservation (23).

The type of the cryo-device may affect the process of vitrification. The carriers, such as cryoloop (24), cryotop (25), open pulled straw (26), and EM grid (27), load a very minimal amount of cryopreservation solutions and increase the rate of cooling that is important in the vitrification process.

Among these devices, cryotop has been successfully applied for ovarian tissue vitrification (25, 28). The majority of cryo-devices in vitrification of isolated ovarian follicles include the open carrier systems (29, 30).

We preferred to use cryolock as a derivative of cryotop. Our results demonstrated that about 84% of follicles were morphologically intact immediately post warming, while 13.6% survived to the end of the IVC interval period in the control group. For normal development of ovarian follicles *in vivo*, a combination of endocrine, autocrine, and paracrine signals growth factor signals is required to work closely.

FGFs are involved in various biological processes during folliculogenesis as follows i. primordial follicle activation, ii. regulation of granulosa and cumulus cell mitosis, apoptosis and glycolysis (31-33), iii. the expression of LH receptors induced by granulosa cells, and iv. proliferation of ovarian germ cells Zhang and Ealy demonstrated that COCs incubated with FGF2 showed an increase in the percentage of oocytes with an extruded polar body as compared to controls, while no significant differences in polar body extrusion rates were detected between FGF2 treatments (34).

Oocyte maturation of IVC method is determined by nuclear maturation and detected by the first polar body (35).

The results of the present study show clearly that FGF-treated follicles produce more MII oocytes; however, we did not observe any positive effect of FGF on survival and ovulation rates of cultured follicles.

FGF is a molecule with multiple functions in the body. In most *in vitro* culture, FGF promotes proliferation of granulosa cells of various species (15, 36-39). Despite of its presence in oocyte and granulosa cells of most follicle stages, the expression level of FGF in different stages is not clear (40-45). In a study by sharma et al. (46), survival, growth, antrum formation and steroidogenesis are stimulated by insulin growth factor-I and bFGF, whereas tumor growth factor-alpha + tumor growth factor -beta inhibited growth and survival of PFs which led to induced oocyte apoptosis in buffalo preantral follicles.

In combination with gonadotropins, FGF-4 increased cumulus expansion and number of metaphase II-stage oocytes in *in vitro* culture (22). It has been reported that EGF increases the proportion of metaphase II stage oocytes of COCs isolated from small follicles (47). In COCs of mouse and pig, EGF-like growth factors is regulated by autocrine mechanism (48, 49). A significant positive EGF on IVM of oocytes was reported in pigs (43, 45). Our finding showed that EGF enhances cumulus expansion and post-thaw COC formation.

Furthermore, expansion of cumulus cells is induced by any changes occurring in level of gonadotropins, growth factors, steroids, and other factors secreted by the oocyte (50). The presence of EGF and a related ligand, transforming growth factor- α (TGF- α), in the human and mouse follicular fluid indicates the participation of EGF in regulation of oocyte maturation (51).

The results of our study showed that the use of 40 ng/ml EGF and FGF simultaneously reduced the number of surviving follicles.

EGF stimulates oocyte maturation through destroying communications between oocyte and cumulus cells (52) or through the signaling pathways promoting oocyte maturation (53).

Conclusion

The inclusion of EGF and FGF at a concentration of 20 ng/ml in mouse leading to vitrified follicle culture system has no effect on follicle survival. Furthermore, 20 ng/ml FGF significantly increases oocyte maturation capacity, where 20 ng/ml EGF only influences ovulation *in vitro*. Combination of FGF and EGF has no effect on survival rate, oocyte maturation and ovulation.

Acknowledgments

This work was a part of thesis of graduate student from the Basic Science Faculty of Islamic Azad University, Damghan Branch and was conducted and funded partially by the Faculty of Medical Sciences, Guilan University of Medical Sciences, Rasht, Iran. There is no conflict of interest in this article.

References

- Oktay K, Buyuk E. The potential of ovarian tissue transplant to preserve fertility. *Expert Opin Biol Ther.* 2002; 2(4): 361-370.
- Bedaiwy MA, Falcone T. Ovarian tissue banking for cancer patients: reduction of post-transplantation ischaemic injury: intact ovary freezing and transplantation. *Hum Reprod.* 2004; 19(6): 1242-1244.
- Gook DA, Edgar DH, Stern C. Cryopreservation of human ovarian tissue. *Eur J Obstet Gynecol Reprod Biol.* 2004; 113 Suppl 1: S41-S44.
- Carroll J, Gosden RG. Transplantation of frozen-thawed mouse primordial follicles. *Hum Reprod.* 1993; 8(8): 1163-1167.
- Cortvrindt R, Smitz J, Van Steirteghem AC. A morphological and functional study of the effect of slow freezing followed by complete in-vitro maturation of primary mouse ovarian follicles. *Hum Reprod.* 1996; 11(12): 2648-2655.
- Kim DH, No JG, Park JJ, Park JK, Yoo JG. Successful in vitro development of preantral follicles isolated from vitrified mouse whole ovaries. *Reprod Dev Biol.* 2012; 36(4): 255-260.
- Shaw JM, Cox SL, Trounson AO, Jenkin G. Evaluation of the long-term function of cryopreserved ovarian grafts in the mouse, implications for human applications. *Mol Cell Endocrinol.* 2000; 161(1-2): 103-110.
- Desai N, AbdelHafez F, Ali MY, Sayed EH, Abu-Ahassan AM, Falcone T, et al. Mouse ovarian follicle cryopreservation using vitrification or slow programmed cooling: assessment of in vitro development, maturation, ultra-structure and meiotic spindle organization. *J Obstet Gynaecol Res.* 2011; 37(1): 1-12.
- dela Peña EC, Takahashi Y, Katagiri S, Atabay EC, Nagano M. Birth of pups after transfer of mouse embryos derived from vitrified preantral follicles. *Reproduction.* 2002; 123(4): 593-600.
- Newton H, Illingworth P. In-vitro growth of murine preantral follicles after isolation from cryopreserved ovarian tissue. *Hum Reprod.* 2001; 16(3): 423-429.
- Adashi EY. The ovarian follicular apparatus. In: Adashi EY, Rock JA, Rosenwaks Z, editors. *Reproductive endocrinology, surgery and technology.* 1st ed. Philadelphia: Lippencott-Raven; 1995: 17-40.
- Eppig JJ, Schroeder AC. Capacity of mouse oocytes from preantral follicles to undergo embryogenesis and development to live young after growth, maturation and fertilization in vitro. *Biol Reprod.* 1989; 41(2): 268-276.
- Spears N, Boland NI, Murray AA, Gosden RG. Mouse oocytes derived from in vitro grown primary ovarian follicles are fertile. *Hum Reprod.* 1994; 9(3): 527-532.
- Quennell JH, Stanton JA, Hurst PR. Basic fibroblast growth factor expression in isolated small human ovarian follicles. *Mol Hum Reprod.* 2004; 10(9): 623-628.
- Bahadori MH, Azarnia M, Ghasemi F, Ghadarjani S, Khojasteh N, et al. Relevance of hepatocyte growth factor and fibroblast growth factor on mouse preimplantation embryo development. *Journal of Reproduction and Contraception.* 2009; 20(4): 195-204.
- Adashi EY, Resnick CE, Croft CS, May JV, Gospodarowicz D. Basic fibroblast growth factor as a regulator of ovarian granulosa cell differentiation: a novel non-mutagenic role. *Mol Cell Endocrinol.* 1988; 55(1): 7-14.
- Gospodarowicz D, Plouet J, Fujii DK. Ovarian germinal epithelial cells respond to basic fibroblast growth factor and express its gene: implications for early folliculogenesis. *Endocrinology.* 1989; 125(3): 1266-1276.
- Herbst RS. Review of epidermal growth factor receptor biology. *Int J Radiat Oncol Biol Phys.* 2004; 59(2 suppl): 21-26.
- Miyazawa k. Role of epidermal growth factor in obstetrics and gynecology. *Obstet Gynecol.* 1992; 79(6): 1032-1040.
- Gómez E, de los Santos MJ, Ruiz A, Tarín JJ, Remohí J, Pellicer A. Effects of epidermal growth factor in the final stages of nuclear and cytoplasmic oocyte maturation in humans. *Hum Reprod.* 1993; 8(5): 691-694.
- Maruo T, Ladines-Llave CA, Samoto T, Matsuo H, Manalo AS, Ito H, et al. Expression of epidermal growth factor and its receptor in the human ovary during follicular growth and regression. *Endocrinology.* 1993; 132(2): 924-931.
- Smitz J, Cortvrindt R, Hu Y. Epidermal growth factor combined with recombinant human chorionic gonadotrophin improves meiotic progression in mouse follicle-enclosed oocyte culture. *Hum Reprod.* 1998; 13(3): 664-669.

23. Segino M, Ikeda M, Hirahara F, Sato K. In vitro follicular development of cryopreserved mouse ovarian tissue. *Reproduction*. 2005; 130(2): 187-192.
24. Lane M, Schoolcraft WB, Gardner DK. Vitrification of mouse and human blastocysts using a novel cryoloop container-less technique. *Fertil Steril*. 1999; 72(6): 1073-1078.
25. Hasegawa A, Hamada Y, Mehandjiev T, Koyama K. In vitro growth and maturation as well as fertilization of mouse preantral oocytes from vitrified ovaries. *Fertil Steril*. 2004; 81 Suppl 1: 824-830.
26. Vajta G, Holm P, Kuwayama M, Booth PJ, Jacobsen H, Greve T, et al. Open Pulled Straw (OPS) vitrification: a new way to reduce cryoinjuries of bovine ovarian and embryos. *Mol Reprod Dev*. 1998; 51(1): 53-58.
27. Martino A, Songsasen N, Leibo SP. Development into blastocysts of bovine oocytes cryopreserved by ultra-rapid cooling. *Biol Reprod*. 1996; 54(5): 1059-1069.
28. Hasegawa A, Mochida N, Ogasawara T, Koyama K. Pup birth from mouse oocytes in preantral follicles derived from vitrified and warmed ovaries followed by in vitro growth, in vitro maturation, and in vitro fertilization. *Fertil Steril*. 2006; 86 Suppl 4: 1182-1192.
29. Bielanski A, Nadin-Davis S, Sapp T, Lutze-Wallace C. Viral contamination of embryos cryopreserved in liquid nitrogen. *Cryobiology*. 2000; 40(2): 110-116.
30. Bielanski A, Bergeron H, Lau PC, Devenish J. Microbial contamination of embryos and semen during long term banking in liquid nitrogen. *Cryobiology*. 2003; 46(2): 146-152.
31. Sugiura K, Su YQ, Diaz FJ, Pangas SA, Sharma S, Wigglesworth K, et al. Oocyte-derived BMP15 and FGFs cooperate to promote glycolysis in cumulus cells. *Development*. 2007; 134(14): 2593-2603.
32. Peluso JJ, Pappalardo A. Progesterone maintains large rat granulosa cell viability indirectly by stimulating small granulosa cells to synthesize basic fibroblast growth factor. *Biol Reprod*. 1999; 60(2): 290-296.
33. Nilsson E, Parrott JA, Skinner MK. Basic fibroblast growth factor induces primordial follicle development and initiates folliculogenesis. *Mol Cell Endocrinol*. 2001; 175(1-2): 123-130.
34. Zhang K, Ealy DA. Supplementing fibroblast growth factor 2 during bovine oocyte in vitro maturation promotes subsequent embryonic development. *Open J Anim Sci*. 2012; 2(2): 119-126.
35. Picton HM. Oocyte maturation in vitro. *Curr Opin Obstet Gynecol*. 2002; 14(3): 295-302.
36. Gospodarowicz D, Bialecki H. The effects of the epidermal and fibroblast growth factors on the replicative lifespan of cultured bovine granulosa cells. *Endocrinology*. 1978; 103(3): 854-865.
37. Gospodarowicz D, Bialecki H. Fibroblast and epidermal growth factors are mitogenic agents for cultured granulosa cells of rodent, porcine, and human origin. *Endocrinology*. 1979; 104(3): 757-764.
38. Lavranos TC, Rodgers HF, Bertonecello I, Rodgers RJ. Anchorage-independent culture of bovine granulosa cells: the effects of basic fibroblast growth factor and dibutyl cAMP on cell division and differentiation. *Exp Cell Res*. 1994; 211(2): 245-251.
39. Roberts RD, Ellis RC. Mitogenic effects of fibroblast growth factors on chicken granulosa and theca cells in vitro. *Biol Reprod*. 1999; 61(6): 1387-1392.
40. Grothe C, Unsicker K. Immunocytochemical localization of basic fibroblast growth factor in bovine adrenal gland, ovary, and pituitary. *J Histochem Cytochem*. 1989; 37(12): 1877-1883.
41. Wordinger RJ, Brun-Zinkernagel AM, Chang IF. Immunohistochemical localization of basic fibroblast growth factor (bFGF) within growing and atretic mouse ovarian follicles. *Growth Factors*. 1993; 9(4): 279-289.
42. Van Wezel IL, Umaphysivam K, Tilley WD, Rodgers RJ. Immunohistochemical localization of basic fibroblast growth factor in bovine ovarian follicles. *Mol Cell Endocrinol*. 1995; 115(2): 133-140.
43. Nandi S, Ravindranatha BM, Gupta PS, Raghu HM, Sarma PV. Developmental competence and post-thaw survivability of buffalo embryos produced in vitro: effect of growth factors in oocyte maturation medium and of embryo culture system. *Theriogenology*. 2003; 60(9): 1621-1631.
44. Das K, Stout LE, Hensleigh HC, Tagatz GE, Phipps WR, Leung BS. Direct positive effect of epidermal growth factor on the cytoplasmic maturation of mouse and human oocytes. *Fertil Steril*. 1991; 55(5): 1000-1004.
45. Abeydeera LR, Wang WH, Cantley TC, Rieke A, Prather RS, Day BN. Presence of epidermal growth factor during in vitro maturation of pig oocytes and embryo culture can modulate blastocyte development after in vitro fertilization. *Mol Reprod Dev*. 1998; 51(4): 395-401.
46. Sharma GT, Dubey PK, Kumar GS. Effects of IGF-1, TGF-alpha plus TGF-beta1 and bFGF on in vitro survival, growth and apoptosis in FSH-stimulated buffalo (*Bubalis bubalus*) preantral follicles. *Growth Horm IGF Res*. 2010; 20(4): 319-325.
47. Sakaguchi M, Dominko T, Yamauchi N, Leibfried-Rutledge ML, Nagai T, First NL. Possible mechanism for acceleration of meiotic progression of bovine follicular oocytes by growth factors in vitro. *Reproduction*. 2002; 123(1): 135-142.
48. Shimada M, Hernandez-Gonzalez I, Gonzalez-Robayna I, Richards JS. Paracrine and autocrine regulation of epidermal growth factor-like factors in cumulus oocyte complexes and granulosa cells: key roles for prostaglandin synthase 2 and progesterone receptor. *Mol Endocrinol*. 2006; 20(6): 1352-1365.
49. Yamashita Y, Kawashima I, Yanai Y, Nishibori M, Richard JS, Shimada M. Hormone-induced expression of tumor necrosis factor alpha-converting enzyme/A disintegrin and metalloprotease-17 impacts porcine cumulus cell oocyte complex expansion and meiotic maturation via ligand activation of the epidermal growth factor receptor. *Endocrinology*. 2007; 148(12): 6164-6175.
50. Buccione R, Vanderhyden BC, Caron PJ, Eppig JJ. FSH-induced expansion of the mouse cumulus oophorus in vitro is dependent upon a specific factor(s) secreted by the oocyte. *Dev Biol*. 1990; 138(1): 16-25.
51. Toretta SA, diZerega GS. Intraovarian regulation of

- follicular maturation. *Endocr Rev.* 1989; 10(2): 205-229.
52. Dekel N, Sherizly I. Epidermal growth factor induces maturation of rat follicle-enclosed oocytes. *Endocrinology.* 1985; 116(1): 406-409.
53. Downs SM. Specificity of epidermal growth factor action on maturation of the murine oocyte and the cumulus oophorus in vitro. *Biol Reprod.* 1989; 41(2): 371-379.
-

Archive of SID