J.Animal and Poultry Prod., Mansoura Univ., Vol.5 (8): 473 - 482, 2014

IMPACT OF INSULIN CONCENTRATION ON *In vitro* MATURATION OF RABBIT OOCYTES.

El - Harairy, M. A.*; Sh. M. Shamiah; M. N. El - Arian* and Hoda A.M. yassen**

*** Anim. Prod. Dept., Fac. Agric., Mansoura University. Egypt**

**** Anim. Prod. Res. Inst., Agric. Res. Center. Egypt**

ABSTRACT

This study aimed to evaluate the effect of adding insulin hormone at different levels (0, 5, 10 and 15 µg/ml) to maturation medium with or without exogenous hormones (FSH, LH and E17β) on the *in vitro* maturation (IVM) of rabbit oocytes. Total of 20 New Zealand White (NZW) rabbit does (5.5-6 mo of age and 2.5 - 3 kg LBW) were used as oocyte donors. Oocytes were recovered from ovaries of slaughtered does using slicing technique. All oocytes with evenly granulated dark ooplasm were matured in TCM-199 supplemented with 6% bovine serum albumin. Eight types of TCM-199, four types without and other four with exogenous hormones supplemented with insulin (0, 5, 10 and 15 µg /ml) were used. Oocytes were fixed and stained for examination after 18 h at 38.5°C, 5% CO2 and high humidity as a maturation period. Results showed significant ($P<0.05$) effect of insulin supplementation on IVM of rabbit oocyte only in terms of oocytes reaching MI, TI+MII and degenerated ones. Percentage of mature oocytes reaching MII was improved by all insulin levels as compared to un-supplemented media (42.1-44.6 vs. 34.1%), but the differences were not significant. Percentage of oocytes reaching both TI+MII increased ($P<0.05$) with insulin supplementation at a level of 5 µg/ml showing the best ($P<0.05$) improvement on oocyte maturation (51.0%) as compared to other insulin levels (45.5-47.6%) or the control medium (39.5%). Nuclear maturation of rabbit oocytes was not affected significantly when FSH, LH and E2 were deleted from the maturation medium, but Percentage of oocytes reaching both T1+MII stages was enhanced in the presence of hormones during the maturation period regardless of whether oocytes were treated with insulin or not. Maturation rate in term of oocytes reaching T1+MII was affected significantly ($P<0.05$) by the interaction between insulin and hormonal addition, reflecting improved percentage of oocytes reaching both T1+MII stages by addition of all insulin levels to hormone-TCM-199 medium. In this respect, insulin addition at a level of 5µg/ml showed the best result (58.7%).

In conclusion, the present study demonstrated that the supplementation of the maturation medium with insulin improves the *in vitro* maturation rate of rabbit oocytes when oocytes are maturated in a defined maturation medium with or without hormones (FSH, LH and E2).

Keywords: Rabbit, oocyte, insulin, gonadotropin, in vitro maturation.

INTRODUCTION

Follicular development is controlled by various factors (Gonadotrophins, steroids, growth factors) of endocrine and paracrine origin (Ireland, 1987 and Webb *et al.,* 1994). Several studies have indicated that insulin and insulin-like growth factor-I (IGF-I) stimulate the proliferation of granulosa cells and the production of progesterone (Gong *et al.,* 1993; Spicer *et al.,* 1993). Therefore, it is expected that insulin and IGF-I have some beneficial effects on *in vitro* maturation of mammalian oocytes. Some studies showed that supplementation of the maturation medium with insulin improved cumulus expansion and oocyte fertilizing ability *in vitro* (Lorenzo *et al.,* 1994

and Webb *et al.,* 1994), but other reports showed that insulin had no significant effect on the fertilization rate or morula formation (Stubbings *et al.,* 1990).

Ocaña-Quero *et al.* (1998) reported that addition of human and bovine insulin to the maturation medium showed a positive effect on *in vitro* maturation, fertilization, and cleavage of bovine oocytes matured *in vitro*, when oocytes were cultured in TCM-199 medium supplemented with fetal calf serum (FCS). Stubbings *et al.* (1990) reported that bovine insulin (1- 1000 ng/ml) has no effect on *in vitro* maturation when bovine oocytes were cultured in TCM-199 medium containing FCS, gonadotrophins and estrogen (E2). Also, Matsui *et al.* (1995) reported that insulin (10 µg /ml) had no effect on the maturation, fertilization and cleavage rates of bovine oocytes. However, it was found that bovine insulin (0.1- 10 µg /ml) enhanced the mitosis of bovine granulosa cells (Spicer *et al.,* 1993), stimulated amino acids transport (Kaye *et al.,* 1986), mitosis (Gardner and Kaye, 1991), and protein synthesis in mouse embryos (Harvey and Kaye, 1988). In this respect, Gong *et al.* (1993) reported that this stimulating effect of insulin on the proliferation of bovine granulosa cells synergistic with gonadotrophins.

Insulin-like growth factor-I (IGF-I) has been located on the luminal epithelium of the rabbit oviduct and uterus at various stages of pregnancy (Herrler *et al.,* 1992), as it has been in other species (Simmen *et al.,* 1995). Therefore, it was postulated an influence of IGF-I on early rabbit embryo development, similar to that on mice (Harvey and Kaye 1992).

Aim of the present study was to establish the effect of adding insulin hormone at different levels (0, 5, 10 and 15 µg/ml) to *in vitro* maturation medium with or without exogenous hormones (FSH, LH and E17β) on the maturation rate of rabbit oocytes *in vitro*.

MATERIALS AND METHODS

This study was carried out at the Laboratory of Physiology and Biotechnology, Animal Production Department, Faculty of Agriculture, Mansoura University**,** in cooperation with the International Livestock Management Training Center (ILMTC), Kafrel-Sheikh Governorate, belonging to the Animal Production Research Institute, Agricultural Research Center, during the period from October 2013 to March 2014.

All chemicals used in this study were purchased from Sigma unless otherwise indicated.

Animals:

Total of 20 New Zealand White (NZW) rabbit does with approximately 5.5-6 months of age and 2.5-3 kg live body weight were used in this study as oocyte doners.

Oocyte collection:

Oocytes were recovered from ovaries of rabbit does immediately after slaughter. Ovaries were removed, washed by warming harvesting medium (Phosphate buffer saline, PBS) supplemented with, 100 IU/ml of sodium penicillin G (Misr Co. for Pharm., Egypt) and, 100 µg/ml streptomycin). Oocytes were collected using slicing technique into glass Petri

dishes containing 4 ml of harvesting medium/dish. Ovaries were held by a forceps and a scalpel blade were made incisions along the whole ovarian surface. Ovaries were washed three times with harvesting medium in dishes containing 2 ml medium for washing and searching oocytes using stereomicroscopy. All oocytes with evenly granulated dark ooplasm were used in this study.

Oocyte maturation:

On the day of maturation, TCM-199 (Egyptian Organization for Biological Product and vaccine, Agoza) was supplemented with 6% of bovine serum albumin (BSA), 20 mMol final concentration of pyruvate and 50 µg/ml gentamicin.

Experimental design:

In this study, eight types of maturation medium (TCM-199), four types without and other four with exogenous hormones supplemented with insulin at level of (0, 5, 10 and 15 µg /ml) were used in maturation of rabbit oocytes *in vitro.*

The pH value of all media were adjusted at 7.3-7.4 and osmolarity at 280-300 mOsmol/kg. The medium was filtrated by 0.22-µm millipore filter (milieux GV, millepore, Cooperation Bedford MOA**)**.

About 100 µl droplets from each prepared medium was placed into sterile Petri dish (30 x 60 mm) and covered by sterile mineral oil. Dishes used in maturation were previously incubated in CO_2 incubator (5% CO_2) at 38.5°C and high humidity for one hour at least for equilibration.

Oocytes were washed three times in PBS + 3% BSA and then in prepared maturation media (TCM-199) to remove substances which prevented maturation. Oocytes were incubated for 18 h at 38.5°C, 5% CO2 and high humidity in Petri dishes containing each type of maturation medium.

Oocytes fixation and staining for examination:

After 18 h as a maturation period, oocytes were washed using PBS containing 1 mg /ml hyaluranidase to remove the cumulus cells. Then, oocytes were washed two times in PBS supplemented with 3% BSA and loaded on clean slide. Slides were placed into fixation solution (3 ethanol: 1 glacial acetic acid) overnight, and then stained with 1 % orcein in 45% acetic acid and examined for maturation stages under phase-contrast microscopy as the following: oocytes with germinal vesicle (GV): chromosomal in disk in cytoplasmic with intact membrane of nuclei; oocytes with germinal vesicle breakdown (GVBD): chromosomal in disk in cytoplasmic but intact membrane of nuclei is breakdown; oocytes at metaphase I (MI), anapahase I (AI), telophase I (TI) and metaphase II (MII), as well as degenerated oocytes (Deg.): Oocytes were vaculated or cytoplasmic shrinked or chromatin condensed.

Statistical analysis:

Data were analyzed using analysis of variance using computer program of SAS (2004) to study the effect of hormonal addition of gonadotrophins, insulin level or their interaction on *in vitro* maturation of rabbit oocytes (2 x 4 factorial design). The significant differences among means were preformed using Duncan Range Test (Duncan, 1955).

RESULTS

Effect of insulin level:

Data presented in Table (1) showed significant (P<0.05) effect of insulin supplementation on IVM of rabbit oocyte only in terms of oocytes reaching MI, TI+MII and degenerated ones. However, oocytes at each of GV, GVD, AI, TI and MII stage were not affected significantly by insulin supplementation. It is of interest to note that percentage of mature oocytes reaching MII was improved by all insulin levels as compared to un-supplemented media (42.1-44.6 vs. 34.1%), but the differences were not significant. When the maturation rate was expressed in term of oocytes reaching both TI+MII, insulin supplementation at a level of 5 µg/ml showed significantly (P<0.05) the best improvement on oocyte maturation (51.0%) as compared to other insulin level (45.5-47.6%) or the control medium (39.5%). The potential role of insulin level supplements on IVM of immature rabbit oocytes, in particular, at a level of 5 µg/ml was reported in bovine oocytes by Dashtizad *et al.* (2010). who demonstrated that presence of 1 or 10 µg/ml insulin in the maturation media showed a positive effect on maturation and cleavage rates of bovine immature oocytes *in vitro*. Also our results are in agreement with previous reports in which addition of insulin to the IVM medium showed a positive effect on *in vitro* development of bovine oocytes cultured in TCM-199 medium supplemented with FCS (Dieleman *et al*., 2002).

Table (1): *In vitro* nuclear maturation of rabbit oocytes as affected by insulin addition to maturation medium.

Insulin level	Oocyte maturation phase							
	GV	GVBD	MI	AI	TI	MII	TI + MII	Deg.
0 µg/ml	1.1	14.5	30.4^{a}	0.0	5.4	34.1	39.5^{b}	14.5^{ab}
5 µg/ml	0.0	13.6	22.4^{ab}	1.1	6.4	44.6	51.0^{a}	11.9^{ab}
10 µg/ml	0.3	12.2	20.7^{b}	1.0	3.0	42.5	45.5^{ab}	20.2^{a}
15 µg/ml	0.0	18.8	25.7^{ab}	0.0	5.6	42.1	47.6^{ab}	8.0^{b}

A and b: Means denoted within the same column with different superscripts are significantly different at P<0.05.

On the other hand, Matsui (1995) showed that addition of insulin at 10µg/ml to the oocyte maturation medium had no effect on the nuclear maturity in bovine. It has been shown that insulin (0.1-10 µg/ml) enhanced the mitosis of bovine granulosa cells and accelerated progression of meiosis in oocytes enclosed with cumulus cells. Both granulosa cells and oocyte normally express insulin receptor (Dashtizad *et al.,* 2010). In accordance with the present results regarding the significant reduction in percentage of degenerated oocytes by 10 µg/ml insulin, Wasielak and Bogacki (2007) found that the incidence of apoptotic DNA degeneration was reduced by addition of insulin, insulin like growth factor I (IGF I) and other growth factors to the maturation medium. Addition of insulin to IVM medium reduced not only the incidence of spontaneous apoptosis in bovine embryos (Augustin *et al*., 2003) but also blocked apoptosis induced by exogenous factors, such as heat shock (Jousan and Hansen, 2004).

It is worthy noting that addition of higher concentration of insulin (10 and 15 µg/ml) did not show positive effect on IVM of rabbit oocytes. This could be due to differences in glucose utilization of oocytes at different maturation stages. Similar finding were reported at different stages of bovine embryo (morula and blastocyst stages) as reported by Harvey and Kaye (1990).

Effect of hormones:

Results presented in Table (2) showed that nuclear maturation of rabbit oocytes was not affected significantly when FSH, LH and E2 were deleted from the maturation medium. The *in vitro* maturation rate of rabbit oocytes in term of oocytes reaching both T1+MII stages was enhanced in the presence of hormones during the maturation period regardless of whether oocytes were treated with insulin or not. These results may suggest that the supplementation of the maturation medium with hormones (FSH, LH and E2) improves the maturation rate.

Table (2): *In vitro* nuclear maturation of rabbit oocytes as affected by hormonal addition to maturation medium.

Hormones	Oocyte maturation phase (%)							
	GV	GVBD	MI	AI	TI	MII	TI+MII	Deg.
Without	0.0	13.5	28.5	0.7	4.1	39.6	43.6	13.7
With	0.7	16.0	21.1	0.4	6.1	42.1	48.1	13.6

In agreement with the present results, (Matsui *et al.,* 1995) reported that insulin had no effects on the nuclear maturation rate, fertilization rate and developmental rate to the blastocyst stage when rabbit oocytes were cultured in TCM-199 supplemented with FSH and E2. In ovine, Moor and Trounson (1977) demonstrated that E2 with FSH and LH was found to be helpful in achieving oocyte maturation *in vitro. Also,* addition of gonadotrophins was found to be essential for the IVM of lamb oocytes (Wahid et al., 1992; O-Brien et al., 1994; Ladda et al., 1997).

In bovine, Goto and Iritani (1990) reported that hormones (E2, LH or FSH) in medium plays an important role in nuclear and cytoplasmic maturation of bovine oocytes cultured in serum-supplemented medium. Downs (1993) reported that gonadotropins stimulate meiotic induction and led to generation of positive factor that acts on oocytes to override the inhibitory influence and to induce GVBD. Saeki et al., (1990) found that commercially available FSH preparations can be used successfully for maturation of bovine follicular oocytes in vitro, which leads to *in vitro* development to the blastocysts.

In buffaloes, Totey et al., (1993) found that addition of hormones (E17β, LH or FSH) alone or in combination with sera further improved the maturation rate, but no differences were observed in maturation rate among the three hormones.

On the other hand, Yang *et al.* (1993) showed high maturation rates to metaphase II-stage (90-95%) with or without the addition of hormones (FSH, 0.5 µg/ml; LH, 5.0 µg/ml and oestradiol, 1.0 µg/ml) to the basic maturation medium (TCM-199 + FCS). However, other authors found that

cumulus cell expansion significantly (P<0.05) decreased by the addition of E2 (Romero-Arredondo and Seidel, 1996 and Gliedt *et al.,* 1996). While, the addition of LH to the maturation medium significantly (P<0.05) enhanced cumulus cell expansion. Also, Beker *et al.* (2002) found that culture of cow cumulus oocyte complexes (COCs) for 22 h in the presence of 1µg/ml oestradiol significantly (P<0.0001) decreased the percentage of metaphase II-stage oocytes as compared to the control group (56.3 and 74.0%, respectively). Moreover, the proportion of oocytes presenting nuclear aberrations was also significantly (P<0.0001) higher in the presence of oestradiol (2.1 vs. 13.3%).

In addition, Accardo *et al.* (2004) found that the highest maturation rate (91.9%) was reached with addition of r-FSH/r- LH to maturation medium. However, no statistical difference was found when this group was compared with the hypophysial Gonadotrpins group (84.0%). However, addition of E2 to the IVM medium was not required when medium already contains follicular fluid (Guler *et al.,* 2000). Recently, Faragi *et al.* (2013) showed that the supplementation of gonadotrpins (PMSG and hCG) to culture media significantly (P<0.05) improved meiotic maturation rate of camel denuded oocytes than that cultured in hormone-free media.

Effect of interaction:

Data shown in Table (3) showed that maturation rate in term of oocytes reaching T1+MII was affected significantly (P<0.05) by the interaction between insulin and hormonal addition, reflecting improved percentage of oocytes reaching both T1+MII stages by the addition of all insulin levels to hormone-TCM-199 media. In this respect, insulin addition at a level of 5 µg/ml showed the best result. However, the *in vitro* maturation rate of rabbit oocytes (T1+MII) in free hormone- TCM 199 was markedly enhanced in the presence of insulin at a level of 15 µg/ml during the maturation period regardless of whether oocytes were treated with hormones or not. These results may suggest that the supplementation of the free hormone-TCM 199 with insulin at higher levels improves the nuclear maturation rate up to 52.4%, but the effect of low level insulin on maturation rate (TI+MII) was remarkable in hormone-TCM 199 medium (58.7%). Therefore, insulin supplementation had beneficial effect on *in vitro* nuclear maturation of rabbit oocytes, regardless of whether oocytes were treated with hormones or not.

The obtained data from the current study indicated an interaction effect between insulin and hormones (FSH, LH and E2) on embryonic stages atartig at M II. Similarly (Dashtizad et al., 2010) showed an interaction between insulin and FSH/or E2 on the bovine COCs. Also, Suzuki *et al*. (2006) reported that stimulating effect of insulin on the proliferation of bovine granulosa cells is synergistic with gonadotrophins. Therefore, the existence of FSH and E2 in the IVM medium might interfere the real effects of insulin on the maturation of bovine cumulus-intact oocytes. Insulin by itself had a positive effect on *in vitro* bovine embryo production system (Dashtizad *et al.,* 2010). Furthermore, insulin increases progesterone and estradiol production by bovine granulosa cells in the presence of FSH (Spicer *et al.,* 1993).

Table (3): *In vitro* nuclear maturation of rabbit oocytes as affected by the interaction between insulin and hormonal addition to maturation medium.

Hormones	Insulin (µg/ml)	Oocyte maturation phase							
		GV	GVBD	MI	AI	TI	MII	TI+MII	Deg.
with	0	2.2	16.5	23.2	0.0	8.8	30.1	38.9	19.1
	5	0.0	11.5	21.2	1.4	8.6	50.2	58.7	7.1
	10	0.7	11.6	18.0	0.0	4.3	47.8	52.1	17.6
	15	0.0	24.4	22.2	0.0	2.8	40.2	42.9	10.5
without	0	0.0	12.5	37.7	0.0	2.0	38.0	40.0	9.9
	5	0.0	15.6	23.7	0.8	4.2	39.1	43.3	16.7
	10	0.0	12.8	23.4	2.1	1.7	37.2	38.9	22.8
	15	0.0	13.2	29.1	0.0	8.4	44.0	52.4	5.4
Significance		NS	NS	NS	NS	NS	*	*	*

NS: not significant. * Significant atP<0.05.

These findings indicated that there may be an interaction between the effects of insulin and the effects of FSH and/or E2 on the cumulus-intact bovine oocytes. Therefore, the presence of FSH and E2 in the maturation medium might have made it difficult to define the effects of insulin on the maturation of bovine cumulus-intact oocytes (Matsui *et al.,* 1995). On the other hand, Stubbings *et al.* (1990) reported that insulin (1-1000 ng/mL) has no effect on *in vitro* maturation when bovine oocytes are cultured in TCM-199 containing gonadotrophins and E2.

In conclusion, the present study demonstrated that the supplementation of the maturation medium with insulin improves the *in vitro* maturation rate of rabbit oocytes when oocytes are maturated in a defined maturation medium with or without hormones (FSH, LH and E2).

REFERANCES

Accardo, C; Datten, M; Pilichi, S; Mara, L; Chessa, B. and Cappai, P. (2004). Effect of recombinant human FSH and LH on in vitro maturation of sheep oocytes, embryo development and viability. Animal Reproduction Science, 81: 77–86.

Augustin, R; Pocar, P; Wrenzycki, C; Niemann, H. and Fischer, B. (2003). Mitogenic and anti-apoptotic activity of insulin on bovine embryos produced in vitro. Reproduction, 126: 91-99.

Beker, A. R.C.L; Colenbrander, B. and Bevers, M.M. (2002). Effect of 17ß-estradiol on the in vitro maturation of bovine oocytes. Theriogenology, 58: 1663-1673.

Dashtizad, M; Haron, A; Yusoff, R; Daliri, M; Hajarian, H; Najari, M; Keng Chee, Y. and Othman, A. M. (2010). Synergistic Effect of Insulin on in vitro Development of Immature Bovine Oocytes. American Journal of Animal and Veterinary Sciences, 5: 258-265.

Dieleman, S.J; Hendriksen, P.J.M; Viuff, D; Thomsen, P.D; Hyttel, P; Knijn, H.M; Wrenzycki, C; Kruip, T.A; Niemann, H; Gadella, B.M; Bevers, M.M. and Vos, P.L. (2002). Effects of in vivo prematuration and in vivo final maturation on developmental capacity and quality of preimplantation embryos. Theriogenology, 57: 5-20.

Downs, S.M. (1993). Factors affecting the resumption of meiotic maturation in mammalian oocytes. Theriogenology, 39: 65-79.

Duncan, D.B. (1955). Multiple Range and Multiple F-Test.Biometrics,11: 1-42.

Faragi, I.M; Girgis, S.M; Zowail, M.E. and Abd El-Hafez, M.A.M. (2013). In vitro Maturation of Camel (*Camelus dromedarius*) Cumulus-Denuded Oocytes. World Applied Sciences Journal 26 (3): 352-359.

Gardner, H.G. and Kaye, P.L. (1991). Insulin increase cell numbers and morphological development in mouse preimplantation embryos in vitro. Reproduction Fertility and Development, 3: 79-92.

Gliedt, D.W; Rosenkrans, C.F; JR; Rorie, R.W; Munyon, A.L; Pierson, J. N; Miller, G.F. and Rakes, J.M. (1996). Effects of media, serum, oviductal cells, and hormones during maturation on bovine embryo development in vitro. J. Dairy Sci., 79: 53-542.

Gong, J.G; McBride, D; Bramley, T.A. and Webb, R. (1993). Effects of recombinant bovine somatotrophin, insulin-like growth factor-I and insulin on the proliferation of bovine granulose cells in vitro. Journal Endocrinology, 139: 67-75.

Goto, K. and Iritani, A. (1990). Symposium 11. Oocytes and embryos in vitro. Chairperson: T.A.M. Kruip (Nether lands). Animal Reproduction Science, 28: 407-413.

Guler, A; Poulin, N; Mermillod, P; Terqui, M. and Cognie, Y. (2000). Effect of growth factor, EGF and IGF-I and estradiol on in vitro maturation of sheep oocyte. Theriogenology, 54: 209-218.

Harvey, M.B. and Kaye, P.L. (1992). Insulin-like growth factor-1 stimulates growth of mouse preimplantation embryos in vitro. Mol. Reprod. Dev., 31:195–199.

Harvey, M.B. and Kaye, P.L. (1990). Insulin increases the cell number of the inner cell mass and stimulates morphological development of mouse blastocysts in vitro. Development, 110: 963-967.

Harvey, M.B. and Kaye, P.L. (1988). Insulin stimulates protein synthesis in compacted mouse embryos. Endocrinology, 122: 1182- 1184.

Herrler, A; Hegele-Hartung, C. and Beier, H.M. (1992). Immunohistological detection of IGF-I in rabbit oviducts and uteri during early pregnancy. Acta Endocrinol., 126(suppl.,4):79 (abstract).

Ireland, J.J. (1987). Control of follicular growth and development. Journal Reproduction & Fertility, 34: 39-541.

Jousan, F. and Hansen, P. (2004). Insulin-like growth factor-I as a survival factor for the bovine preimplantation embryo exposed to heat shock. Biol. Reprod., 71: 1665-1670.

Kaye, P.L., Pemble, L.B. and Hobbs, J.G. (1986). Protein metabolism in preimplantation mouse embryos. Prognosis Clinical Biology Research, 217B: 103-106.

Ladda, S; Bogliolo, L; Calvia, P; Leoni, G.and Naitana, S. (1997). Meiotic progression and developmental competence of oocytes collected from juvenile and adult ewes. J. Reprod. Fertil., 109: 73-78.

Lorenzo, P.L; M.J; Illera, J.C. Illera and M. Illera. (1994). Enhancement of cumulus expansion and nuclear maturation during bovine oocyte maturation in vitro by the addition of epidermal growth factor and insulin-like growth factor-I. Journal Reproduction&Fertility,101:697-701.

Matsui, M; Takahashi, Y; Hishinuma, M. and Kanagawa, H. (1995). Effects of supplementation of the maturation media with insulin on in vitro maturation and in vitro fertilization of bovine oocytes. Japanese Journal Veterinary Research, 43: 145-153.

Moor, R.M. and Trounson, A.O. (1977). Hormonal and follicular factors affecting maturation of sheep oocytes matured in vitro and their subsequent developmental capacity. J. Reprod. Fertil., 49: 101-109.

O-Brien, J.K; Rhodes, S.L; Maxwell, W.M.C. and Evans, G. (1994). Hormonal requirement for in vitro maturation of sheep oocytes. Theriogenology, 41: 266.

Ocaña-Quero, J.M; M; Pinedo-Merlín, M; Ortega-Mariscal, Moreno-Millán, y M. (1998). Influence of human and bovine insulin on in vitro maturation, fertilization and cleavage rates of bovine oocytes. Arch. Zootec., 47: 85-93.

Romero-Arredondo, A. and Seidel, G.E. Jr. (1996). Effects of follicular fluid during in vitro maturation of bovine oocytes on in vitro fertilization and early embryonic development. Biol. Reprod., 55: 1012-1016.

Saeki, K; Hoshi, M; Leibfried-Rutledge, M.L. and First, N. L. (1990). In vitro fertilization and development of bovine oocytes matured with commercial available follicle stimulating hormone. Theriogenology, 34: 1035-1039.

SAS (2004). SAS/Stat. User's Guide Static's, Ver., 6.06 4th Ed. SAS Institute Inc. Cary, NC.

Simmen, R.C.M; Green, M.L. Simmen, F.A. (1995). IGF system in preimplantation uterus and embryonic development. In: Dey SK (ed.), Molecular and Cellular Aspects of Preimplantation Processes. New York: Springer Verlag; 185–203.

Spicer, L.J; Alpizar, E. and Echternkamp, S.E. (1993). Effects of insulin, insulin-like growth factor I and gonadotrophins on bovine granulosa cell proliferation, progesterone production in vitro. Journal Animal Science, 71: 1232-1242.

Stubbings, R.B; Liptrap, R.M; Betteridge, K.J; Walton, J.S; Armstrong, D.T. and Basrur, P.K. (1990). Requirements for bovine oocyte maturation in vitro. Reproduction Domestic Animal, 25: 158-166.

Suzuki, H. and Saito, Y. (2006). Cumulus cells affect distribution and function of the cytoskeleton and organelles in porcine oocytes. Reprod. Med. Biol., 5: 183-194.

Totey, S. M; Pawshe, C. H. and Singh, G. P. (1993). Effects of bull and heparin and sperm concentration on in vitro fertilization of buffalo (Bubalus bubalis) oocytes matured in vitro.Therriogenology,30:887-898.

Wahid, H; Monaghan, P. and Gordon, I. (1992). In vitro maturation (IVM) of sheep follicular oocytes. J. Reprod. Fertil., 9: 52.

Wasielak, M. and Bogacki, M. (2007). Apoptosis inhibition by IGF-I during in vitro maturation of bovine oocytes. J. Reprod. Dev., 53: 192-202.

Webb, R; Gong, J.G. and Bramley, T.A. (1994). Role of growth hormone and intrafollicular peptides in follicle development in cattle. Theriogenology, 41: 25-30.

Yang, X; Jiang, S. and Foote, R.H. (1993). Bovine oocyte development following different oocyte maturation and sperm capacitation Procedures. Molecular Reproduction and Development, 34: 94-100.

تأثير تركيز الأنسولين على الإنضاج المعملى لبويضات الأرانب

مصطفى عبد الحليم الحرايري*، شريف مغاورى شاميه، محمد نجيب العريان*و هدى عبده محمد ياسين***

*** قسم انتاج الحيوان ، كلية الزراعة ، جامعة المنصورة**

**** معهد بحوث الانتاج الحيوانى، وزارة الزراعة**

هدفت هذه الدراسة إلى تقييم اضافة الأنسولين بمستويات مختلفة (صفر ، ٥ ، ١٠ ، ١٥ ميكروجرام / مل) إلـــى بيئة الإنضاج المعملي مع أو بدون إضافة هرمونات خارجيــة (FSH, LH, E17β) على عملية الإنضاج المعملى لبويضات الأرانب. استخدم فى هذه الدراسة ٢٠ أنثى أرنب نيوزيلاندي أبيض (متوسط عمر ٥.٥ – ٦ شهر ومتوسط وزن حى ٢.٥ -٣ كجم) كأمهات معطيه للبويضات. تم إسترداد البويضات من مبايض الإناث المذبوحة بطريقة التشريح. تم إستخدام كل البويضات ذات السيتوبلازم المتماثل والمتجانس فى عملية الإنضاج المعملى. وتم إستخدام بيئة زراعة الأنسجة ١٩٩ المضاف إليها ٦% ألبيومين سيرم الابقار فى عملية الإنضاج المعملى. استخدمت ثمانية أنواع من بيئة زراعة الأنسجة أربعة منها مع اضافة الهرمونات الخارجية والأربعة الآخرى بدون اضافة الهرمونات الخارجية مع اضافة أربع مستويات من هرمون الانسولين (صفر ، ٥ ، ١٠، ١٥ميكروجرام /مل) فى كل منها. تم تثبيت البويضات وصبغها بعد ١٨ ساعة من الإنضاج على درجة حرارة ٣٨.٥ م و٥% ثانى أكسيد كربون وذلك لمعرفة مرحلة النضج النووى. أظهرت النتائج وجود تأثير معنوي بإضافة الأنسولين إلى بيئة الإنضاج المعملي لبويضات الأرانب التى وصلت لمرحلة الطور الإستوائى الأول و الطور النهائى الأول+ الطور الإستوائى الثانى والبويضات المضمحلة. تحسنت نسبة البويضات التى وصلت لمرحلة النضج النووى (الطور الإستوائى الثانى) مع كل تركيزات الأنسولين المضافة بالمقارنة بالبيئة التى لا تحتوى على الأنسولين حيث كانت (٤٢.١-٤٤.٦ مقابل ٣٤.١%) ولكن كان هذا التحسن غير معنوى. زادت نسبة البويضات التى وصلت لمرحلة الطور النهائى الأول+ الطور الإستوائى الثانى معنوياً بإضافة الأنسولين بتركيز ٥ ميكروجرام / مل حيث كانت ٥١% بالمقارنة بباقى التركيزات ١٠ و ١٥ ميكروجرام / مل حيث كانت ٤٥.٥ و ٤٧.٦% على التوالى أو المجموعة القياسية ٣٩.٥%. لم يتأثر النضج النووى لبويضات الأرانب معنوياً عند عدم إضافة هرمونات (FSH, LH ,E2) لبيئة الإنضاج. أيضاً تحسنت نسبة البويضات التى وصلت لمرحلة الطور النهائى الأول+ الطور الإستوائى الثانى بوجود الهرمونات أثناء مرحلة النضج النووي بغض النظر عن المعاملة بالأنسولين من عدمه. تأثرت معنوياً نسبة البويضات التى وصلت لمرحلة النضج النووى (الطور النهائى الأول+ الطور الإستوائى الثانى) نتيجة للتداخل بين إضافة الأنسولين والهرمونات إلى بيئة الإنضاج. حيث كانت أعلى نسب للبويضات التى وصلت لمرحلة النضج فى البيئة المحتويه على الهرمونات والمضاف إليها الإنسولين بالتركيزات المختلفة. وكانت أحسن النتائج فى البيئة المحتوية على الهرمونات والمضاف إليها الأنسولين بتركيز ٥ ميكروجرام /مل (٥٨.٧%).

نستنتج من هذه الدراسة ان اضافة الأنسولين إلى بيئة الإستزراع المعملي مع أو بدون إضافة هرمونات (FSH,LH, E2) تحسن من معدل نضج بويضات الأرانب.