



INFLUENCE OF ADDING ESSENTIAL AND NON-ESSENTIAL AMINO ACIDS TO CULTURE MEDIUM ON CLEAVAGE AND BLASTOCYST FORMATION RATES OF RABBIT EMBRYOS AT 4- AND 8-CELL STAGES

Sh.M. Shamiah*, Sara, F. Fouda**, G. E. Younan* and H. Zaghloul***

* *Anim. Prod. Res. Ins., Agric. Res. Cent. Dokki, Giza, Egypt*

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

*** *High Institute for Agricultural Co-operation, Shoubra, Egypt*

Received: 01/11/2015

Accepted: 20/11/2015

ABSTRACT: This study aimed to evaluate the effect of essential (EAAs) and non-essential (NEAAs) amino acids (AAs) at levels of 25 and 50 μ l/ml, respectively, on in vitro developmental potential of rabbit embryos at 4- and 8-cell stages. Total of 12 sexual mature New Zealand White (NZW) rabbit does and 3 fertile NZW bucks were used in this study. All does and bucks were kept under similar conditions of feeding and management. Does were superovulated by eCG and GnRH (Receptal). Embryos were collected by flushing at 4 and 8-cell stages after about 32 and 40 h post- GnRH injection and mating, respectively. Embryos were in vitro cultured in TCM-199 supplemented with EAAs and NEAAs at a level of 25 (M1) or 50 (M2) μ l/ml from each as compared to control (CM). Results showed slight increase in number of cleaved embryos and those reached blastocyst stage of embryos in vitro cultured at 4-cell as compared to those at 8-cell stage in spite of increasing number of embryos at 8-cell than at 4-cell stage. Cleavage and blastocyst rates were higher ($P \geq 0.05$) for embryos at 4-cell than at 8-cell stage (81.25 and 65.17% vs. 73.27 and 56.03%). Proportion of cleavage and blastocyst formation rates were higher (82.27 and 65.82%, $P < 0.05$) in M2 than in M1 (78.67 and 60%) and CM (70.27 and 55.40%). Supplementation of M1 with AAs increased ($P \geq 0.05$) the proportion of cleavage and blastocyst formation rates as compared to CM. The effect of interaction between embryonic stage and amino acid supplementation on cleavage and blastocyst rates was not significant, reflecting the highest cleavage and blastocyst rates of embryos cultured at 4-cell stage M2 (87.17 and 71.79%). In conclusion, developmental competence of rabbit embryos at 4-cell and 8-cell stages to reach blastocyst stage was improved by supplementation of in vitro culture medium (TCM-199) with essential and non-essential amino acids at a level of 50 μ l for each.

Key words: : Rabbit, amino acids, culture medium, embryonic stage, , cleavage, blastocyst.

Corresponding author: ggezzate@yahoo.com)

INTRODUCTION

Embryos grown in vitro reduced viability post-transfer (Bavister, 1995). Optimal culture conditions are required for developmental competence of embryo viability post-transfer. Under suboptimal condition of culture, there is evidence for reduced embryo viability. Normal growth and differentiation of mammalian embryos in vitro during the pre-implantation period appear to be dependent upon the availability of appropriate metabolic substrates (Sanyal and Frederick, 2005).

The development of simple chemically defined media has allowed the analysis of specific requirements of the pre-implantation embryo as it develops from the 1-cell to the blastocyst stage. Earlier reports mentioned that amino acids (AAs) could play an important role in embryo development in rat (Kishi et al., 1991) and rabbit (Kane and Foote, 1970) embryos. It was reported that AAs, including glutamine, phenylalanine, methionine and isoleucine supported the first cell division of hamster embryos (Gwatkin and Haidri, 1973).

Several studies indicated the role of AAs in pre-implantation development of mouse (Lane and Gardner., 1997 a and b), and human (Gardner and Lane, 1997) embryos, although some AAs can stimulate, while others can inhibit the embryo development (Bavister and McKiernan, 1993).

Amino acids (AAs) have been vital roles in protein and nucleotides synthesis and signaling molecule biosynthesis (Katchadourian et al., 1994; Wu and Morris, 1998). Also, AAs control trophectoderm differentiation (Martin and Sutherland, 2001) and basement membrane formation between primitive endoderm and ectoderm (Biggers, et al., 2000). Moreover, AAs are considered as osmo- and pH regulators and antioxidant factor as reported by Dawson et al. (1998).

Supplementation of culture media with AAs has been shown to benefit pre-implantation embryo development in several species (Devreker et al., 2001). Also, some authors concluded that there were marked effects of essential (EAAs) and non-essential (NEAAs) amino acids on embryonic physiology. Blastocyst development was improved in many species by culture in relatively simple media containing optimized concentrations of AAs (Rieger et al., 1992; Swain et al., 2002). In buffalo, Badr (2009) found that development of the early cleavage stages was stimulated by NEAAs and glutamine, while development beyond 3 days was stimulated by a combination of NEAAs and EAAs and glutamine. Furthermore, AAs present in high concentrations in female reproductive tract fluids and have been shown to improve pre-implantation mammalian embryo development in vitro (Bavister, 1995; Gardner and Lane, 1997).

In sheep, Willadsen (1979) found that embryos at 2-, 4-, and 8-cell stages show no difference in producing monozygotic twins. However, rabbit embryos could develop in the absence of exogenous energy substrates up to the morula stage, but required AAs for blastocyst formation and hatching (Kane and Foote, 1970). Little information are available about the effect of AAs on rabbit embryo differentiation and development in vitro. Therefore, aim of the current study was to evaluate the effect of EAAs and NEAAs at levels of 25 and 25 or 50 and 50 µl/ml, respectively, on in vitro developmental potential of rabbit embryos at 4- and 8-cell stages.

MATERIALS AND METHODS

This study was conducted at Biotechnology Laboratory, Faculty of Agriculture, Mansoura University in co-operation with Animal Peroduction Research Institute, agricultural Research

Center. All chemicals used in this study were purchased from Sigma (Madrid, Spain).

Total of 12 New Zealand White (NZW) rabbit does of approximately 5-6 months of age, 2.75-3.5 kg live body weight (LBW) and within 1st - 2nd parity were used in this study as donors of embryos. Also, 3 fertile NZW bucks, about 9 months of age and 3.75 kg LBW were used for natural mating. All does and bucks were kept under similar conditions of feeding and management. All rabbits were fed ad libitum on a commercial pelleted diet and housed individually in metal cages provided with feeders and water nibble in each cage.

All rabbit does were superovulated by an intramuscular injection with 40 IU/kg of Equine Chorionic Gonadotropin (eCG) (Folligon, Intervet International B.V., Boxmeer, Netherlands), followed by 0.2 ml GnRH analogue (Receptal, Intervet International B.V., Boxmeer, Holland) immediately after natural mating. Embryos were recovered by flushing each oviduct per doe using phosphate buffer saline (PBS) according to the developmental stage of embryo. Embryos at 4 and 8-cell stages were collected after about 32 and 40 h post-GnRH injection, respectively.

Phosphate buffer saline (PBS) medium (Gordon, 1994) was supplemented with 2 mg/ml Bovine Serum Albumin (BSA), 22 mM Na-pyruvate (final concentration) and 50 µg/ml Gentamycin sulphate. All media were adjusted to pH value of 7.2-7.4 using pH-meter and to osmolarity level of 280-300 mOsmol/kg using osmometer. Then, the medium was filtered by 0.22 µm millipore filter (milieux GV, millipore, Cooperation Bedford MOA).

All flushings were collected in sterile plastic Petri dishes and embryos were washed three times with PBS, counted and evaluated for viable and un-viable embryos under a stereomicroscope (20–40 X. Only the viable embryos were used in this study.

Viable embryos at 4-cell and 8-cell stages were cultured in vitro by placing into 4-well Petri dishes containing (0.5 ml per well) basic medium (TCM-199, The Egyptian Organization for Biological Products and Vaccine- Agouza, Egypt) supplemented with 22 mM Na-pyruvate (final concentration), 4 mg/ml BSA and 50 µg/ml Gentamycin sulphate as control medium (M1). The basic medium was supplemented with solution containing a combination (Louis, France) of essential (EAAs) and non-essential amino acids (NEAAs) at a level of 25 µl/ml from each (M2, low level of EAAs+NEAA) or at a level of 50 µl/ml from each of EAAs and NEAAs (M3, high level of EAAs+NEAAs). Media were covered with sterile mineral oil and incubated at 38.5°C and 5% CO₂ with 95% humidity for intact embryos at 4-cell and 8-cell stages for 96 and 72 h, respectively. Each medium was replacement every 24 h.

Embryos were examined by inverted microscope (200 X) for searching development of 4- and 8-cell embryos to blastocyst stage.

The experiment was replicated 3 times for determining the effect of embryonic stage (4- vs. 8-cell), amino acid level supplementation (0, low and high) and their interaction on cleavage rate and blastocyst production rate. Data were statistically analyzed by analysis of variance (ANOVA, factorial 2 x 3) using SAS (2004) after arcsine transformation. Duncan's Multiple Range Test was followed for test the significant differences among means (Duncan, 1955).

RESULTS AND DISCUSSION

Embryo recovery rate:

Total of 260 embryos were collected from the 12 superovulated donor does, 132 at 4-cell and 128 at 8-cell stages. Average total number of embryos was 21.7/doe (23.1 per donor), 335 of them were considered as morphological intact embryos at the 4 and 8 cell stages (22.0 for

4-cell and 21.3 for 8-cell/doe), with viability rate of 84.8 and 90.6%, respectively (Table 1).

Effect of embryonic stage:

Data presented in Table 2 show slight increase in number of cleaved embryos and those reached blastocyst stage of embryos in vitro cultured at 4-cell as compared to those at 8-cell stage in spite of increasing number of embryos at 8-cell than at 4-cell stage. This resulted in higher cleavage and blastocyst rates for embryos at 4-cell than at 8-cell stage (81.25 and 65.17% vs. 73.27 and 56.03%), but the differences were not significant. Such findings may be attributed to that the timing of human embryonic genome activation between the 4- and 8-cell stages (Braude et al., 1988) explains the flexibility of the cells at the 4-cell stage. However, Willadsen (1979) reported that embryos at 2-, 4-, and 8-cell stages show no difference in producing monozygotic twins in sheep.

Effect of amino acids addition to in vitro culture medium:

Data in Table 3 show that, apart from embryonic stage, culture medium supplemented with combination of EAAs and NEAAs at high level significantly ($P < 0.05$) increased the proportion of cleavage and blastocyst formation rates (82.27 and 65.82%) as compared to low AAs level (78.67 and 60%) and control media (70.27 and 55.40%). However, culture medium supplemented with EAAs and NEAAs at low level increased the proportion of cleavage and blastocyst formation rates as compared to control medium, but did not differ significantly from that in high AAs level and control media.

It is worthy noting that increasing level of EAAs and NEAAs from 25 to 50 µl/ml for each significantly ($P < 0.05$) improved in vitro cultured of rabbit embryos apart from embryonic stage. In accordance with the obtained impact of AA on in vitro culture of rabbit embryos, the beneficial effect of AAs addition to the

culture media compared with defined media without AAs supplementation was established (Rezaei and Chian, 2005). Similarly, Huang et al. (2004) reported that AAs had certain specific functions in the development of pig embryos to the morula/blastocyst stage. Addition of NEAAs and glutamine stimulated cleavage, differentiation of inner cell mass and fetal development after transfer. Several authors reported effects of EAAs and NEAAs on embryonic physiology, and morula/blastocyst development was improved in many species by culture in relatively simple media containing optimized concentrations of AAs (Rieger et al., 1992; Swain et al., 2002). Also, Gardner et al. (1994) reported that AAs addition to culture media reduced the percentage of embryos arrested during culture and stimulated both cleavage and hatching by the increase of endogenous amino acid pool sizes and/or protein synthesis. It was found that culture of mouse embryos from the 2-cell stage with NEAAs and glutamine significantly increased blastocyst formation, cell number, hatching, and post-implantation development (Lane and Gardner, 1994) and stimulated the development of the mouse embryo by increasing the time of the first three cleavage divisions (Lane and Gardner, 1997c). In buffalo, Badr (2009) found that development of the early cleavage stages was stimulated by NEAAs and glutamine, while development beyond 3 days was stimulated by a combination of EAAs, NEAAs and glutamine. Recently, Shamiah (2014) reported increase of cleavage and morula/blastocyst rates of blastomeres isolated from rabbit embryos at early stages in vitro cultured in medium (TCM-199) supplemented with combination of EAAs and NEAAs at level of 50 µl/ml for each.

In addition, specific amino acid transporters are present on the membranes of oocytes and embryos and a supply of AAs for protein synthesis is essential for normal embryo growth (Van Winkle and

Dickinson, 1995). In vitro, culture media components and culture conditions can affect and even modulate the development of mammalian embryo (Hardy et al., 1989). Rabbit embryo has a requirement for AAs during development from the zygote to the blastocyst (Al-Luhbi and Al-Bashan, 2013). Research has largely focused on the availability of energy substrates and their role in stimulating embryo development. Rabbit zygote and embryo have been one of the most responsive to development in vitro. All the pre-implantation stages have undergone development in culture (Maurer, 1978). In early report, Naglee et al. (1969) pointed out that early development of the rabbit embryo was equivalent after culture in the presence of NEAAs (media also contained glutamine), and EAAs, as well as vitamins and trace elements. Generally, regulation of osmotic pressure is a critical factor for embryo development (Liu and Foote, 1996). Osmolytes maintain proper cell function by protecting protein structure, particularly the structure of enzymes. In general AAs play important physiological functions including; synthesis of proteins and nucleotides (Katchadourian et al., 1994) nutrition and energy provision (Houghton et al., 2002),

osmo-regulation and protection against oxidative stress (Dawson et al., 1998), pH regulation (Edward et al., 1998) signaling molecule biosynthesis (Wu and Morris, 1998), trophoblast differentiation (Martin and Sutherland, 2001) and basement membrane formation between primitive endoderm and ectoderm (Biggers et al., 2000).

Such results indicated that rabbit embryos at different stages may show variation in their requirements for AAs. Content of AAs in mouse embryos decreased when embryos were cultured in media lacking AAs resulting in reduction of embryo viability (Van Winkle and Dickinson, 1995). The addition of AAs to synthetic industrial medium enriched with potassium (Lawitts and Biggers, 1991), enhanced the development of mouse embryos in vitro to a similar level as embryos grown in vivo (Ho et al., 1995).

In conclusion, developmental competence of rabbit embryos at 4-cell and 8-cell stages to reach blastocyst stage was improved by supplementation of in vitro culture medium (TCM-199) with essential and non-essential amino acids at a level of 50 µl for each.

Table (1): Total number and average number of embryos recovered at different embryonic stages from superovulated rabbit does

Item	Total	Embryonic stage	
		4-cell	8-cell
Number of superovulated does	12	6	6
Total number of recovered embryos	260	132	128
Number of total embryos/doe	21.7	22.0	21.3
Number of intact embryos	228	112	116
Number of total embryos/doe	19.0	18.7	19.3
Viability rate of embryos	87.7	84.8	90.6

Cleavage and blastocyst rates:

Table(2): Effect of embryonic stage on cleavage and blastocyst rates of embryos cultured in vitro

Item	Embryonic stage	
	4-cell	8-cell
Total embryos	112	116
Number of cleaved embryos	91	85
Cleavage rate	81.25	73.27
Number of blastocyst	73	65
Blastocyst rate	65.17	56.03

Table (3): Effect of amino acids supplementation to in vitro culture medium of embryos at 4- and 8-cell stages on cleavage and blastocyst rates

Item	Culture medium		
	Control (0 level)	AAs (low level)	AAs (high level)
Total embryos	74	75	79
Number of cleaved embryos	52	59	65
Cleavage rate	70.27 ^b	78.67 ^{ab}	82.27 ^a
Number of blastocyst	41	45	52
Blastocyst rate	55.40 ^b	60.00 ^{ab}	65.82 ^a

a and b: Means denoted within the same row with different superscripts are significantly different at ($P < 0.05$).

AA (low level): 25+25 µl/ml EAAs and NEAAs. AA (high level): 50+50 µl/ml EAAs and NEAAs.

Effect of interaction between embryonic stage and amino acid addition:

Along with the insignificant effect of embryonic stage and the significant effect of AAs supplementation on cleavage and blastocyst rates, their interaction was not significant, reflecting the highest cleavage and blastocyst rates of embryos cultured at 4-cell stage (87.17 and 71.79%).

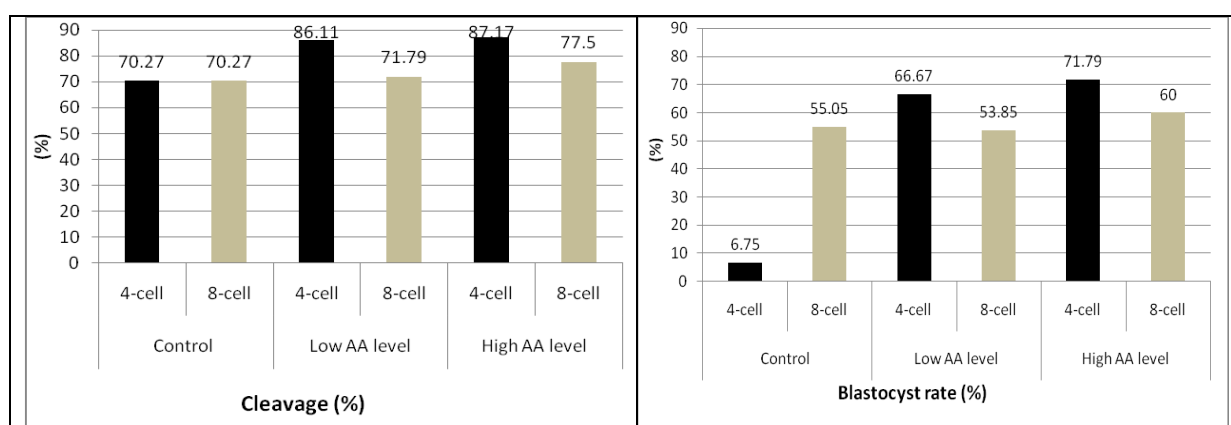


Fig. 1. Cleavage and blastocyst rates of embryos at 4- and 8-cell stages in vitro cultured in medium supplemented with amino acid (control, low AA level: 25+25 µl/ml EAAs and NEAAs and high AA level: 50+50 µl/ml (EAAs and NEAAs)).

REFERENCES

- Al-Luhbi, F. M. and Al-Bashan, M. M. (2013).** Toward a Rabbit-Optimized Culture Medium: Impact of Supplementation with Amino Acids on Early Development of in vitro Cultured Rabbit Embryos. *World Journal of Zoology* 8 (4): 344-353.
- Badr, M.R. (2009).** Effects of supplementation with amino acids on in vitro buffalo embryo development in defined cultured media. *Global Veterinaria*, 3 (5): 407-413.
- Bavister, B.D. (1995)** Culture of preimplantation embryos: facts and artifacts. *Hum. Reprod. Update*, 1, 91-148.
- Bavister, B.D. and McKiernan, S.H. (1993)** Regulation of hamster embryo development in vitro by amino acids. In Bavister, B. (ed.), *Preimplantation Embryo Development*. Springer-Verlag, New York, 57 pp.
- Biggers, J.D.; McGinnis, L. and Raffin, M. (2000).** Amino acids and preimplantation development of the mouse in protein-free potassium simplex optimized medium. *Biology of Reproduction*, 63: 281-293.
- Braude, P.; Bolton V. and Moore, S. (1988).** Human gene expression first occurs between the four-and eight-cell stages of preimplantation development. *Nature*, 332:459-461.
- Dawson, K.M. Collins J.L. and Baltz, J.M. (1998).** Osmolarity-dependent glycine accumulation indicates a role for glycine as an organic osmolyte in early preimplantation mouse embryos. *Biology of Reproduction*, 59: 225-22.
- Devreker, F.; Hardy, K.; Van den Bergh, M.; Vannin, A.S.; Emiliani, S. and Englert, Y. (2001).** Amino acids promote human blastocyst development in vitro. *Human Reproduction* 16, 749-756.
- Duncan, D. B., (1955).** Multiple range and multiple F. Test biometrics.; 11: 1-42.
- Edwards, L.J.; Williams D.A. and Gardner, D.K. (1998).** Intracellular pH of the mouse preimplantation embryo: amino acid act as buffers of intracellular pH. *Human Reproduction*, 13: 3441-3448.
- Gardner, D.K. and Lane, M. (1997).** Culture and selection of viable blastocysts: a feasible proposition for human IVF? *Hum. Reprod. Update*, 3: 367-382.
- Gardner, D.K., Lane, M.; Spitzer, A. and Batt, P.A. (1994).** Enhanced rates of cleavage and development for sheep zygotes cultured to the blastocyst stage in vitro in the absence of serum and somatic cells: amino acids, vitamins and culturing embryos in groups stimulate development. *Biol. Reprod.*, 50: 390-400.
- Gordon, L. (1994).** Laboratory production of cattle embryos CAB International Wallingford. UK.
- Gwatkin, R.B. and Haidri, A.A. (1973)** Requirements for the maturation of hamster oocytes in vitro. *Exp. Cell Res.*, 76, 1-7.
- Hardy, K.; Hooper, M.A.K.; Handyside, A.H.; Rutherford, A.J.; Winston, R.M.L. and Leese, H.J. (1989)** Non-invasive measurement of glucose and pyruvate uptake by individual human oocytes and preimplantation embryos. *Hum. Reprod.*, 4: 188-191.

- Ho, Y.; Wiggelsworth, K.; Eppig, J.J. and Schultz, R.M. (1995)** Preimplantation development of mouse embryos in KSOM: augmentation by amino acids and analysis of gene expression. *Mol. Reprod. Dev.*, 41: 232-238.
- Houghton, F.D.; Hawkhead, J.A.; Humpherson, P.G.; Hogg, J.E.; Balen, A.H.; Rutherford, A.J. and Leese, H.J. (2002).** Non-invasive amino acid turnover predicts human embryo developmental capacity. *Human Reproduction*, 17: 99–1005.
- Huang, Y.J.; Presicce, G.A.; Gasparini, B. Yang, B.Z. Liang, X.W. Zhang, X.F. Chen, M.T. Li, Z.Q. and Huang, F.X. (2004).** Researches on ovum pick-up and in vitro fertilization in buffalo. *China Anim. Husband. Vet. Med.*, 11-22.
- Kane, M.T. and Foote, R.H. (1970)** Culture of two-and four -cell rabbit embryos to the expanding blastocyst stage in synthetic media. *Proc. Soc. Exp. Biol. Med.*, 113: 921–925.
- Katchadourian, C.; Joly, C. and Menezo, Y. (1994).** Interactions in glycine and methonine uptake, conversion and incorporation into proteins in the preimplantation mouse embryo. *Zygote*, 2: 301-306.
- Kishi, J., Noda, Y., Narimoto, K. (1991)** Block to development in cultured rat one-cell embryos is overcome using medium HECM-1. *Hum. Reprod.*, 6: 1445–1448.
- Lane, M. and Gardner, D.K. (1997a).** Nonessential amino acids and glutamine decrease the time of the first three cleavage divisions and increase compaction of mouse zygotes in vitro. *J. Assist. Reprod. Genet.*, 14: 398-403.
- Lane, M. and Gardner, D.K. (1997b).** Differential regulation of mouse embryo development and viability by amino acids. *J. Reprod. Fertil. Dev.*, 109: 153-164.
- Lane, M. and Gardner, D.K. (1997c)** Differential regulation of mouse embryo development and viability by amino acids. *J. Reprod. Fertil.*, 109: 153- 164.
- Lane, M. and Gardner, D.K. (1994)** Increase in postimplantation development of cultured mouse embryos by amino acids and induction of fetal retardation and exencephaly by ammonium ions. *J. Reprod. Fertil.*, 102: 305-312.
- Lawitts, J.A. and Biggers, J.D. (1991).** Optimization of mouse embryo culture media using simplex methods. *J. Reprod. Fertil.*, 91: 543-556.
- Liu, Z. and R.H. Foote, (1996).** Sodium chloride, osmolyte and osmolarity effects on blastocyst formation in bovine embryos produced by in vitro fertilization (IVF) and cultured in simple serum-free media. *J. Assist. Reprod. Genet.*, 13: 562-568.
- Martin, P.M and Sutherland A.E. (2001).** Exogenous amino acids regulate trophectoderm differentiation in the mouse blastocyst through an mTOR- dependent pathway. *Developmental Biology*, 240: 182-193.
- Maurer, R.R. (1978).** Advances in rabbit embryo culture. In: C. Joseph and J.R. Daniel, (eds), *Methods in Mammalian Reproduction*, United Kingdom Edition published by: Academic Press, Inc. (London) LTD., London, pp: 259-272.
- Naglee, D.; Maurer, R.R. and Foote, R.H. (1969).** In: *Advances in rabbit embryo culture* by: Ralph, R. Maurer, In: C. Joseph and J.R. Daniel, (eds), *Methods in Mammalian Reproduction*, United Kingdom Edition published by:

- Academic Press, Inc. (London) LTD., London, pp: 259-272.
- Rezaei, N. and R. Chian, 2005.** Effects of essential and non-essential amino acids on in-vitro maturation, fertilization and development of immature bovine oocytes. Iranian Journal of Reproductive Medicine, 3: 36-41.
- Rieger, D.; Loskutoff, N.M. and Betteridge, K.J. (1992).** Developmentally related changes in the metabolism of glucose and glutamine by cattle embryos produced and co-cultured in vitro . Journal of Reproductive and Fertility, 95: 585-595.
- Sanyal, M. K. and Frederick, N. (2005).** In vitro development of the mammalian embryo. Journal of Experimental Zoology, 2: 235–251.
- Shamiah, Sh. M. (2014).** In vitro developmental potential of blastomeres separated from 4- and 8-cell rabbit embryos and cultured in medium supplemented with amino acids. Site of published: J. Animal and Poultry Prod., Mansoura Univ., Vol. 5 (9): 537-547.
- Statistical Analysis Systems (SAS).** SAS User's Guide. Cary, NC: Statistical Analysis Systems Institute Inc; 2004.
- Swain, J. E.; Bor Mann, C. L.; Clark, S. G.; Walters, E. M.; wheeler, M. B. and kisher, R. L.(2002).** Use of energy substrates by various stage preimplantation pig embryos produced in vivo and in vitro . Reprod.,123: 253-260.
- Van Winkle L.J. and Dickinson, H.R. (1995). Differences in amino acid content of preimplantation mouse embryos that develop in vitro versus in vivo: in vitro effects of five amino acids that are abundant in oviductal secretions. Biol Reprod 52: 96–104.
- Willadsen, S.M. (1979).** A method for culture of micromanipulated sheep embryos and its use to produced mono-zygotic twins. Nature, 278: 298–300.
- Wu, G. and Morris, S.M. (1998).** Arginine metabolism: nitric oxide and beyond. Biochemical Journal, 336: 1-6.

الملخص العربي

تأثير إضافة الأحماض الأمينية الأساسية وغير الأساسية الى بيئة الزراعة على معدل الانقسام وتكوين البلاستوسيست أجنة الارانب في مرحلة ٤ و ٨ خلايا.

*شريف مغاوى شامية - **سارة فكرى فودة - *جورج عزت يونان - ***حلمى زغول

*معهد بحوث الإنتاج الحيوانى- مركز البحوث الزراعية الدقى ، الجيزة ، مصر.

* قسم انتاج الدواجن- كلية الزراعة - جامعة المنصورة- مصر .

***معهد التعاون الزراعى العالى - شبرا- مصر .

تهدف هذه الدراسة لتقييم تأثير إضافة الأحماض الأمينية الأساسية وغير الأساسية بمستويات ٢٥+٢٥ ميكروليتر/ملليتر (البيئة ١) أو ٥٠+٥٠ ميكروليتر/ملليتر (البيئة ٢) على التطور المعمل لاجنة الارانب في مرحلة ٤ أو ٨ خلايا. تم استخدام ١٢ من امهات الارانب النيوزيلاندى الناضجة و ٣ من ذكور الارانب من نفس النوع فى هذه الدراسة. تم حقن الامهات بهرمون PMSG ثم حقنها بالريستال وتلقيحها طبيعيا ثم تجميع أجنة الارانب في مرحلة ٤ و ٨ خلايا بعد ٣٢-٤٠ ساعة من الحقن بالريستال والتلقيح طبيعيا، على التوالي. تم زراعة الاجنة فى بيئة زراعة الانسجة-١٩٩ مضافا اليها (البيئة ١) و (البيئة ٢) ومقارنتها بالكنترول (بدون اضافات). وقد اظهرت النتائج أن:

- ١- زاد عدد الاجنة المنقسمة التى وصلت الى مرحلة البلاستوسيست غير معنويا وذلك للاجنة المنزرعة معمليا فى مرحلة ٤ خلايا مقارنة بالخلايا فى مرحلة ٨ خلايا على الرغم من زيادة عدد الاجنة فى مرحلة ٨ خلايا عن مرحلة ٤ خلايا قبا للزرع.
 - ٢- كان معدل الانقسام والوصول لمرحلة البلاستوسيست أعلى معنويا للاجنة فى مرحلة ٤ خلايا عن مرحلة ٨ خلايا (٨١,٢٥ و ٦٥,١٧ % مقارنة ٧٣,٢٧ و ٦٥,٠٣ %).
 - ٣- كانت نسبة الانقسام ومعدل تكوين البلاستوسيست أعلى معنويا فى البيئة ٢ (٨٢,٢٧ و ٦٥,٨٢ %) عن البيئة ١ (٧٨,٦٧ و ٦٠ %) والكنترول (٧٠,٢٧ و ٥٥,٤٠ %).
 - ٤- أدت إضافة الأحماض الأمينية الى البيئة ١ الى زيادة نسبة الانقسامات ومعدل تشكيل البلاستوسيست مقارنة بالكنترول.
 - ٥- كان تأثير التداخل ما بين المرحلة الجنينية وإضافة الاحماض الامينية على معدل الانقسام ومرحلة البلاستوسيست غير معنوى والتى عكست ارتفاع معدل الانقسام والوصول لمرحلة البلاستوسيست للاجنة المنزرعة فى مرحلة ٤ خلايا فكانت (٨٧,١٧ و ٧١,٧٩ %).
- ونستخلص من هذه الدراسة ان معدل تطور الاجنة فى مرحلتى ٤ و ٨ خلايا ووصولها الى مرحلة البلاستوسيست تحسنت بإضافة الاحماض الامينية الأساسية و غير الأساسية بتركيز ٥٠ ميكروليتر/ملليتر لكل منها.