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In vitro Maturation of Rabbit Oocytes as Affected by Donor Age and Type of Serum From Mated Does in Maturation Medium

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ABSTRACT

This study was designed to examine the effect of the age of rabbit and addition of serum at different reproductive status (pre-, at and post-mating doe serum) in maturation medium (TCM-199) as compared as hormonal addition (10 IU eCG and 20 IU hCG/ml) on *in vitro* maturation of rabbit oocytes. Results show that frequency distribution of oocytes at MII stage was higher ($P<0.05$) for oocytes recovered from old (59.58%) than young (51.69%) rabbits. Frequency distribution of oocytes at MI stage showed an opposite trend, being significantly ($P<0.05$) lower for oocytes recovered from old (9.58%) than the small (14.89%). Frequency distribution of oocytes at MII was the highest (62.11%, $P<0.05$) for oocytes *in vitro* maturated in maturation medium supplemented with post-mating serum, but did not differ significantly from those *in vitro* matured in media supplemented with hormones (57.54%) or pre-mating serum (55.87%), while oocytes matured with mating serum showed significantly the lowest values (46.78%). Frequency distribution of oocytes at MI stage showed an opposite trend to that at MII. The interaction between age of oocyte donors and type of serum was not significant on frequency distribution of oocytes at all maturation stages. Such effect was reflected in higher maturation rat (oocyte at MII) for oocytes matured with Post-mating serum for young or old doners, but *in vitro* maturation rate was the highest (67.5%) for oocytes recovered from older doners and *in vitro* matured in medium supplemented with post-mating serum. Results of the current study revealed that the highest percentage of oocytes at metaphase-II (matured oocytes) and the lowest percentage of degenerated oocytes were obtained when rabbit oocytes were recovered at older ages and cultured in TCM-199 supplemented with serum from post-mating doe serum or hormones (eCG and hCG).

Keywords: *Rabbit, age, doe serum, oocyte, maturation.*

INTRODUCTION

The oocyte is arrested in prophase I of meiosis, whereas oocyte growth occurs primarily in pre-antral follicles. Subsequently, under the action of follicle stimulating hormone (FSH), follicles are selected and undergo an increase in size and granulosa cells specialization into cumulus and mural granulosa cells. During the period between luteinizing hormone (LH) surge and ovulation (final stage of mature follicles, the oocytes resume meiosis to complete their nuclear and cytoplasmic maturation. Exogenous gonadotrophins such as FSH and LH can stimulate these processes (Findlay, 2003).

During in vitro maturation (IVM) of animal oocytes, different serum types are required to add to tissue culture medium (Motlagh *et al.,* 2008; Hegab *et al.,* 2009). These types of serum are important due to its hormonal contents, proteins such as globulin and futuin, and trace nutrients (Hsu *et al.,* 1987). In this respect, Thompson (2000) mentioned that balance of the osmolarity and a free radical scavenger action occurred by IVM medium supplementation with albumin as sera additives. In this respect, maturation media were supplemented with fetal calf serum or estrous sheep serum (Ghasemzadeh-Nava and Tajik, 2000), human serum (Thompson *et al.,* 1992), mare serum (Motlagh *et al.,* 2008), buffalo estrus serum (Totey *et al.,* 1993), and estrus cow serum (Ocana-Quero *et al.,* 1999). Rabbits are induced ovulator animals, where ovulation occurs only at mating. Rabbits exhibit follicular cycles with successful development and regression (Elias *et al*., 1984). Around mating/ovulation, gonadotrophins concentration in blood of does may differ according to time of mating. Maturation media of rabbit oocytes was supplemented with rabbit serum (Gordon, 1994), this serum was randomly taken without limiting age or reproductive stage of does, but no available information in the literature on the effect of serum collected from rabbit at different reproductive status on IVM of rabbit oocytes.

Therefore, the present study was designed to investigate the effect of adding different serum types collected from rabbit pre-, at and post-mating in maturation media as compared as hormones on *in vitro* maturation of oocytes recovered from rabbit does at young or old ages.

MATERIALS AND METHODS

The experimental work of this study was conducted at the Physiology and Biotechnology Laboratory, belonging to department of Animal Production, Faculty of Agriculture, Mansoura University.

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

Animals:

A total of twenty four rabbit does from New Zealand white (NZW) breed was divided into two groups. Does in the 1st group (young does pre-1st parity, n=12) with 2.32 kg live body weight (LBW) and 5.1 months old, while those in the 2nd group (old does, 3-5 parities, n=12) with 3.50 kg LBW and nearly one year old. All does were kept under similar conditions of feeding and management in a private farm. They were housed individually in metal cages (40 x 50 x 60 cm). Feeders and water nipple for drinking were found in each cage. *Ad libitum* feeding on a commercial pelleted diet was allowed for all rabbit does.

Oocyte collection:

Oocyte harvesting medium used in this study was phosphate buffer saline (PBS) prepared according to Gordon (1994). Oocytes were recovered from ovaries of rabbits in both groups after slaughtering. Ovaries were removed, washed by harvesting medium (PBS) supplemented with 100 IU/ml from sodium Penicillin G, (Misr Co. for Pharm. Egypt) and 100 µg/ml from Streptomycin. Oocyte collection was carried out by

slicing technique. Ovaries were held by a forceps and incised along the whole ovarian surface by a scalpel blade. Oocytes were collected in 4 ml of harvesting medium in glass Petri dishes.

After collection, oocytes were washed three times with harvesting medium, and placed in dishes containing 2 ml medium for searching and evaluating oocytes using stereomicroscopy. Only complete cumulus oocytes (COCs), oocytes with ≥3 layers of cumulus cells, were used in this study.

***In vitro* maturation (IVM):**

Rabbit oocytes recovered from both rabbit groups were *in vitro* maturated by rabbit doe serum (RDS) taken from rabbits pre-, at and 12 h post-mating. Natural mating was performed from rabbit does with red vulva, and blood was collected from ear vein of each doe pre-mating, immediately post-mating and 12 h post-mating).

After collection, blood was double centrifuged at 3000 rpm for 15 min, then clear serum was aspirated by pasture pipette and transported in another sterile centrifuge tubes. These tubes were incubated in water bath at 56°C for 30 min. (heat-inactivated serum) and left to cool. The prepared serum was placed into eppendorf and frozen until usage.

Tissue culture medium (TCM-199, powder) was dissolved in deionized double distilled water and 50 µg/ml gentamicin was added to the medium. On the day of maturation, TCM-199 medium stock was supplemented with 20 mmol final concentration of pyruvate, 100 IU/ml of Penicillin G, 100 µg/ml of Streptomycin. Four maturation media containing 20% of three types of serum (pre-, at and post-mating) as well as hormonal addition of 10 IU eCG/ml (Foligon, Invert International BV, Boxmeer, Netherland) and 20 IU hCG/ml (Epifasi, Egyptian Int. Pharmaceutical industries Co., Egypt).

All media were adjusted at pH of 7.3-7.4 and osmolarity of 280-300 mOsmol/l. Media were filtrated by 0.22-µm millipore filter (milieux GV, millepore, Cooperation Bedford MOA (Shamiah, 2004).

Sterile Petri dishes (60 mm) containing each type of prepared medium (100 µl droplets in each dish) and covered by sterile mineral oil were previously incubated in CO_2 incubator (5% CO_2) at 38.5°C and high humidity for one hour at least for equilibration. PBS supplemented with 3% Bovine Serum Albumin (BSA) was used for washing oocytes for three times. To remove substances which prevented maturation, oocytes were also washed in each prepared maturation media thereafter. Dishes containing oocytes in maturation medium were incubated at 38.5°C, 5% CO_2 and high humidity for 18 h.

To remove the cumulus cells surrounding oocytes, PBS supplemnted with one mg/ml hyaluranidase was used for washing of oocytes after maturation. PBS supplemented with 3% BSA was used for oocyte washing two times. Oocytes were loaded on clean slides, which placed into fixation solution (3 ethanol: 1 glacial acetic acid) overnight, stained with 1% orcein in 45% acetic acid and examined under phase-contrast microscopy. The examined ooytes were calssified into five categories as the following: mature oocytes at metaphase II stage with and polar body exhausted in previtilline space (M II); oocytes at germinal vesicle stage (GV) with chromosomal in disk in cytoplasmic with intact membrane of nuclei; oocytes at germinal vesicle breakdown stage (GVBD) with chromosomal in disk in cytoplasmic but intact membrane of nuclei is breakdown; oocytes at metaphase I stage (MI) and degenerated oocytes (DO) (Shamiah, 2004).

Statistical analysis:

Data were analyzed using analysis of variance as factorial design (2 rabbit ages x 4 maturation media) using computer program of SAS (2000). The significant differences among group means were set at $P<0.05$ and preformed by Duncan Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

***In vitro* maturation of rabbit oocytes:**

Effect of rabbit age:

Frequency distribution of oocytes at different maturation stages as affected by rabbit age is presented in Table (1). Analysis of variance revealed that only frequency distribution of oocytes at metaphase-I (MI) and metaphase-II (MII) was affected significantly ($P<0.05$) by rabbit age. However, oocytes at germinal vesicles (GV), germinal vesicles breakdown (GVB) and degenerated (DO) stages were not affected significantly by rabbit age (Table 1).

Results show that frequency distribution of oocytes at MII stage was significantly ($P<0.05$) higher for oocytes recovered from old (59.58%) than young (51.69%) rabbits. However, frequency distribution of oocytes at MI stage showed an opposite trend, being significantly ($P<0.05$) lower for oocytes recovered from old (9.58%) than the small (14.89%).

Table (1): Oocytes at different stages after *in vitro* maturation as affected by rabbit doe age.

Rabbit	Total	GV		GVB		MI		MII		DO	
age	oocytes	n	%	n	%	n	%	n	%	n	%
Young	356	27	7.58	40	11.24	53	14.89[a]	184	51.69[b]	52	14.61
Old	334	29	8.68	31	9.28	32	9.58[b]	199	59.58[a]	43	12.87

a and b: Means denoted within the same column with different superscripts are significantly different at P<0.05. GV: Germinal vesicles. GVB: Germinal vesicles breakdown. MI: Metaphase I. MII: Metaphase II.

The differences in *in vitro* maturation of oocytes as affected by rabbit age may mainly age-related variation in fertility, follicular recruitment and the growthas affected by phase of the estrous cycle and hormonal stimulation, developmental competence of oocytes. In bovine, developmental competence of oocytes from pre-pubertal heifers was improved with age with a remarkable increase of the potential to cleave

between 7 and 9 months of age (Young *et al.* (1998). In older cows, Malhi *et al.* (2005 and 2008) found that fewer 4-5 mm follicles recruited into waves produced lower peak numbers of 6-8 mm follicles and ovulatory follicles with small diameter. Also, higher rate of embryos at blastocyst stage were observed *in vitro* in cows than in heifers using COCs originating from slaughterhouse-derived ovaries (Rizos *et al.,* 2005). Generally, the developmental capacity of oocytes seems to decrease with advancing age, and their quality according to morphological criteria seems to remain relatively stable in aging cows (Katska and Smorag, 1984).

On the other hand, Su *et al.* (2009) indicated that *in vitro* developmental capacity of cow oocytes obtained by ovum pickup (OPU) showed the highest cleavage and blastocysts rates in young cows (12 months), followed by middle (7-8 years) aged and older cows (15 years).

However, no differences in the numbers of COCs or in the cleaved and blastocyst embryos were found between Holstein-Friesian (HF) heifers and early- or mid-lactating multi-parous cows after twice weekly OPU (Roth *et al.,* 2008). Also, during IVM of oocytes from different age groups of crossbred beef heifers, Rizos *et al.* (2005) found no differences the oocyte developmental competence or in formation of embryos at blastocyst stage. Moreover, no differences in the number of COCs obtained and in the *in vitro* blastocyst rates between cows 1-3 years old and older cows with abattoir-derived ovaries from individual animals of different breeds (Mermillod *et al.*, 1992).

Effect of type of maturation medium:

Effect of type of serum (adding hormones or serum of mated does) on *in vitro* maturation rate of rabbit oocytes is shown in Table (2). Effect of type of serum was significant (P<0.05) only on oocytes at MI and MII stages. However, the effect on oocytes at GV, GVBD and DO stages was not significant.

Results showed that frequency distribution of oocytes at MII was significantly (P<0.05) the highest (62.11%) for oocytes *in vitro* maturated in maturation medium supplemented with post-mating serum, but did not differ significantly from those *in vitro* matured in media supplemented with hormones (57.54%) or pre-mating serum (55.87%), while oocytes matured with mating serum showed significantly the lowest values (46.78%). On the other hand, frequency distribution of oocytes at MI stage showed an opposite trend to that at MII (Table 2).

Table (2): Oocytes at different stages after *in vitro* maturation as affected by type of maturation medium.

Type of medium	N	GV		GVB		MI		MII		DO	
		n	%	n	%	n	%	n	%	n	%
With hormones	179	10	5.59	15	8.38	27	15.08^{a}	103	57.54^{ab}	24	13.41
Pre-mating serum	179	15	8.38	18	10.06	21	11.73^{ab}	100	55.87^{ab}	25	13.97
Mating serum	171	17	9.94	22	12.87	23	13.45^{ab}	80	46.78^{b}	29	16.96
Post-mating serum	161	14	8.70	16	9.94	14	8.70^{b}	100	62.11^{a}	17	10.56

a and b: Means denoted within the same column with different superscripts are significantly different at P<0.05. GV: Germinal vesicles. GVB: Germinal vesicles breakdown. MI: Metaphase I. MII: Metaphase II. N: Total oocytes.

In much of the early research on maturation *in vitro* (IVM) of rabbit oocytes, serum was utilized as either the sole constituent or a major constituent of the media. Blood serum was slightly superior when directly compared during IVM of oocytes and their, subsequent fertilization (Cross and Brinster, 1970). Also, beneficial effects of serum supplementation have been demonstrated during maturation of oocytes *in vitro* on subsequent fertilization and development in mice (Cross, 1973). In rabbits, Kane (1985) reported that the blood serum may be contaminated with several extraneous peptides, energy substrates, and growth factors. In this respect, Shea *et al*. (1976) showed that supplementation of TCM-199 with serum of rabbit had beneficial effect on IVM of rabbit oocytes more than BSA supplementation.

The obtained results indicated beneficial effects of using pre- and post-mating doe serum or supplementation of hormones to maturation medium as compared to doe serum at mating, being the highest for post-mating doe serum. Such improvement in IVM of rabbit oocytes by post-mating doe serum may attributed to that the serum contains many substances, including enzymes, hormones and various other proteins, any of which might enhance rabbit oocytes maturation (Shea *et al*., 1976). In sheep, Rao *et al*. (2002) found that percentages of maturation rates were 77, 86, 76, 82 and 58% for oocytes cultured with 20% of different types of serum, bovine embryonic fluid, estrous sheep serum, ovine follicular fluid, granulose cell culture, and control, without serum, respectively. In bovine, Mingoti *et al.* (2002) demonstrated that cumulus cells of COCs are able to secrete estradiol and progesterone in culture systems for *in vitro* maturation, and this steroidgenesis is modulated by the steroids progesterone, testosterone and estradiol. Expansion of cumulus cells surrounding bovine oocytes was altered in response to FSH and/or LH in semi-defined medium, while cumulus expansion was not related to rates of cleavage or subsequent embryonic development *in vitro*.

According to the previous findings, addition of hormones to maturation medium increased IVM of rabbit oocytes. The effects of hormonal addition such as LH on cumulus expansion can be explained by as little as 1 part per 10,000 contaminations with FSH (Choi *et al.,* 2001). Both FSH and LH enhanced fertilizability and developmental ability of bovine oocytes matured *in vitro* (Romero-Arredondo and Seidel, 1996). The lowest IVM of rabbit oocytes with mating doe serum may be related to higher level of E2, because Gliedt *et al.* (1996) found that cumulus cell expansion significantly (P<0.05) decreased by the addition of E2. 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 COCs for 22 h in the presence of 1 µg/ml estradiol significantly (P<0.0001) decreased the percentage of MII oocytes as compared to the control group (56.3 and 74.0%, respectively). Generally, FSH preparations as commercial product could be successfully used for IVM of follicular bovine oocytes, leading to *in vitro* development of embryos to the blastocyst stage (Saeki *et al.,* 1990).

In addition, Accardo *et al.* (2004) found the highest maturation rate (91.9%) of ovine oocytes with addition of r-FSH/r-LH to maturation medium, but no statistical difference was found when this group was compared with the hypophysial gonadotrophins group (84.0%). In contrast to the present results, Chohan and Hunter (1998) found no differences in nuclear maturation and degenerated rate among different maturation media (TCM-199 with four sera types: control, 10% normal buffer estrus serum, 10% heat inactivated and normal buffalo fetal serum.

Effect of interaction between rabbit age and type of serum:

The interaction between age of oocyte donors and type of serum was not significant on frequency distribution of oocytes at all maturation stages. Such effect was reflected in higher maturation rat (oocyte at MII) for oocytes matured with post-mating serum for young or old doners, but *in vitro* maturation rate was the highest (67.5%) for oocytes recovered from older doners and *in vitro* matured in medium supplemented with post-mating serum (Table 3).

Table (3): Oocytes at different stages after *in vitro* maturation as affected by the interaction between rabbit age and type of maturation medium.

Rabbit age	Type of medium	N	Oocyte stage									
			CV		GVB		MI		MII		DO	
			n	%	n	%	n	%	n	%	n	%
Young	A	95	6	6.32	8	8.42	17	17.89	50	52.63	14	14.74
	B	89	8	8.33	10	10.42	14	14.58	49	51.04	15	15.63
	C	71	5	5.95	13	15.48	14	16.67	39	46.43	13	15.48
	D	82	8	9.88	9	11.11	8	9.88	46	56.79	10	12.35
Old	A	84	4	4.76	7	8.33	10	11.90	53	63.10	10	11.90
	B	93	7	8.43	8	9.64	7	8.43	51	61.45	10	12.05
	C	84	12	13.79	9	10.34	9	10.34	41	47.13	16	18.39
	D	87	6	7.50	7	8.75	6	7.50	54	67.50	7	8.75

N: total number of oocytes. A: With hormones. B: Pre-mating serum. C: Mating serum. D: Post-mating serum.

CONCLUSION

Results of the current study revealed that the highest percentage of oocytes at metaphase-II (matured oocytes) and the lowest percentage of degenerated oocytes were obtained when rabbit oocytes were recovered at older ages and cultured in TCM-199 supplemented with serum from post-mating doe serum or hormones (10 IU eCG and 20 IU hCG/ml). These results suggest that serum collected from post-mated does may use as an alternative for hormones during in vitro maturation of rabbit oocytes.

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.

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.

Chohan, K.R. and Hunter, A.G. (1998). Effect of serum supplementation in maturation medium nuclear maturation of water buffalo oocytes. Animal Biotechnology, 9: 201-202.

Choi, Y.H; Carnevale, E.M; Seidel, G.E; and Squires, E.L. (2001). Effects of gonadotropins on bovine oocyte matured in TCM-199. Theriogenology, 56: 661-670.

Cross, P.C. (1973). The role of cumulus cells and serum in mouse oocytes. J. Reprod. Fertil., 3:298-307.

Cross, P.C. and Brinster, R. L. (1970). *In vitro* development of mouse oocytes. Bioi. Reprod., 3:298-307.

Duncan, D.B. (1955). Multiple range and multiple F-test biometrics, 11: 1-42.

Elias, E.; Bedrack, E. and Yagil, R. (1984). Peripheral blood levels of progesterone in femal camels during various reproductive status. Gen. Comp. Endocr., 53: 235-240.

Findlay J.K. (2003). Endocrine regulation of meiosis-local and systemic hormonal influences. In: Trounson, A.O., Gosden, R.G., editors. Biology and pathology of the oocyte. Role in fertility and reproductive medicine. Cambridge: Cambridge University Press, pp 113–9.

Ghasemzadeh-Nava, H. and Tajik, P. (2000). *In vitro* maturation of ovine follicular oocytes in different concentrations of fetal calf serum and estrous sheep serum. Theriogenology, 53: 435.

Gliedt, D.W.; Rosenkrans, C.F; 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.

Gordon, L. (1994). Laboratory production of cattle embryos. CAB International Wallingfored. UK.

Hegab, A.O.; Montasser, A.E.; Hammam, A.M.; Abu El-Naga, E.M.A. and Zaabel, S.M. (2009). Improving *in vitro* maturation and cleavage rates of buffalo oocytes. Anim. Reprod., 6 (2): 416-421.

Hsu, C.J.; Holmes, S.D. and Hammond, J.M. (1987). Ovarian epidermal growth factor-like activity. Concentrations in porcine follicular fluid during follicular enlargement. Biochem. Biophys. Res. Commun., 147: 242-247.

Kane, M. T. (1985). A molecular weight extract of bovine serum albumin stimulates rabbit blastocyst cell division and expansion *in vitro*. J. Reprod. and Fertil., 73 : 147- 150.

Katska, L. and Smorag, Z. (1984). Number and quality of oocytes in relation to age of cattle. Animal Reproduction Science, 7: 451--460.

Malhi, P.S.; Adams, G.P. and Singh, J. (2005). Bovine model for the study of reproductive aging in women: follicular, luteal, and endocrine characteristics. Biol. Reprod., 73(1): 45-53.

Malhi, P.S.; Adams, G.P.; Mapletoft, R.J. and Singh, J. (2008). Superovulatory response in a bovine model of reproductive aging. Anim. Reprod. Sci., 109 (1-4): 100-109.

Mermillod, P.; Wils, C.; Massip, A. and Dessy, F. (1992). Collection of oocytes and production of blastocysts *in vitro* from individual slaughtered cows. *J. Reprod. Fertil.,* 96(2): 717-723.

Mingoti, G.Z; Garcia, J.M. and Rosa-e-Silva, A.A.M. (2002). Steroidogenesis in cumulus cells of bovine cumulus-oocyte- complexes matured in *vitro* with BSA and different concentrations of steroids. Animal Reproduction Science, 69 : 175-186.

Motlagh, M. K.; Shahneh, A. Z.; Daliri, M.; Kohram, H. and Ghragozlou, F. (2008). *In vitro* maturation of sheep oocytes in different concentrations of mare serum. African J. of Biotechnology, 7 (18): 3380-3382.

Ocana-Quero, J.M.; Pinedo Merlin, M.; Ortega, M.M. and Millan, M.M. (1999). The effect of different sera and bovine serum albumin fraction (BSA) on *in vitro* maturation of immature bovine oocytes. Arch.Zootec, 48:167-174.

Rao, B.S; Naidu, K.S; Amarnath, D; Vagdevi, R; Rao, A.S; Brahmaiah, K.V. and Rao, V.H. (2002). *In vitro* maturation of sheep oocytes in different media during breeding and non-breeding seasons. Small Ruminant Research, 43: 31-36.

Rizos, D.; Burke, L.; Duffy, P.; Wade, M.; Mee, J.F.; O' Farrell, K. J.; Macsiurtain, M.; Boland, M.P. and Lonergan, P. (2005). Comparisons between nulliparous heifers and cows as oocyte donors for embryo production *in vitro*. Theriogenology, 63(3): 939-949.

Romero-Arredondo, A. and Seidel, GE 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.

Roth, Z.; Inbar, G. and Arav, A. (2008). Comparison of oocyte developmental competence and follicular steroid content of nulliparous heifers and cows at different stages of lactation. Theriogenology, 69(8): 932-939.

SAS (2000). SAS Institute Inc. SAS Users's Guide, Statistics, Cary, NC.

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.

Shamiah, S. M. (2004). Studies on *in vitro* fertilizing in Egyptian buffaloes. Ph. D. Thesis, Faculty of Agriculture, Mansoura University, Egypt.

Shea, B.F.; Bekar, R.D. and Latour, J.P.A. (1976). Maturation *in vitro* of rabbit follicular oocytes. Candian. J. Anim. Sci., 56:377-381.

Su, L.; Yang, S.; He, X.; Li, X.; Ma, J.; Wang, Y.; Presicce, G. and Ji, W. (2009). Effect of Donor Age on the Developmental Competence of Bovine Oocytes Retrieved by Ovum Pick Up. Reprod. Domest. Anim.

Thompson, J.G. (2000). *In vitro* culture and embryo metabolism of cattle and sheep embryos – a decade of achievement. Anim. Reprod. Sci., 30: 273 -280.

Thompson JGE; Simpson, A.C.; Pugh, P.A.; Tervit, R.H. (1992). *In vitro* development of early sheep embryos is superior in medium supplemented with human serum compared with sheep serum or human serum albumin. Anim. Reprod. Sci., 29: 61-68.

Totey, S.M.; Pawshe, C.H. and Singh, G.P. (1993). Effects of bull and heparin and sperm concentrations on *in vitro* fertilization of buffalo (*Bubalus bubalis*) oocytes matured *in vitro*. Theriogenology, 39: 887-898.

Young, L.E., Sinclair, K.D. and Wilmut, I. (1998). Large offspring syndrome in cattle and sheep. Rev. Reprod., 3(3): 155-163.

تأثير عُمرِ الأم المعطية للبويضات ونوعِ السيرم على الإنضاج المعملي لبويضات الأرانب.

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أجريت هذه الدراسةِ لمعرفة تأثيرِ عُمرِ الأرنبة وإضافةِ سيرم من مراحل فسيولوجية مختلفةِ (قَبْلَ، وعند وبَعْدَ التلقيح) في بيئة الإنضاج (TCM-199) مُقارنة بإضافة هرمونات (10 IU/ml eCG, 20 IU/ml hCG) على الإنضاج المعملي لبويضات الأرانب. وأظهرت النَتائِجُ أن التوزيع النسبي للبويضات في مرحلةِ الطور الاستوائي الثاني (M11) كَانَ أعلى عند معنوية ($P<0.05$) للبويضات المستردة مِنْ الأرانب الكبيرة (٥٩.٥٨ %) عن مِنْ الصغيرةِ (٥١.٦٩ %). وعلى العكس من ذلك التوزيع النسبي للبويضات في مرحلةِ الطور الاستوائي الأول (M1) كَانَ منخفض بشكل ملحوظ عند معنوية ($P<0.05$) للبويضات المستردة مِنْ الأرانب الكبيرة (٩.٥٨ %) عن مِنْ الصغيرةِ (١٤.٨٩ %). التوزيع النسبي للبويضات في مرحلةِ الطور الاستوائي الثاني (M11) كَانَ أعلى عند معنوية ($P<0.05$) للبويضات التي تم إنضاجها في بيئة مضاف إليها سيرم بَعْدَ التلقيح (٦٢.١١ %), لكن لَمْ يكن هناك اختلاف معنوي للتي تم إنضاجها في بيئة مضاف إليها هرمونات (٥٧.٥٤ %) أو سيرم قَبْلَ التلقيح (٥٥.٨٧ %)، بينما البويضات التي تم إنضاجها في بيئة مضاف إليها سيرم عند التلقيح كانت الأقل بمعنوية (٤٦.٧٨ %). التوزيع النسبي للبويضات في مرحلةِ الطور الاستوائي الأول (M1) كانت على العكس من اتجاه (M11). التفاعل بين عُمرِ الأم المعطية للبويضات ونوعِ السيرم لم يكن معنوي في التوزيع النسبي للبويضات عند كل مراحلِ الإنضاج. مثل هذا التأثيرِ يعُكِسَ أن أعلى معدل إنضاج للبويضات (البويضات عند M11) كان مع السيرم بعد التلقيح للأعمار الكبيرة والصغيرة ، لكن كان أعلى معدل إنضاج (٦٧.٥ %) عندما استرداد البويضات من الأرانب الكبيرة في العمر إنضاجها في بيئة مضاف إليها سيرم بَعْدَ التلقيح. تشير نَتائِجَ الدراسةِ الحاليةِ بأنَ النسبة المئويةَ الأعلى للبويضات التي وصلت إلى مرحلةِ الطور الاستوائي الثاني (M11) والنسبة المئوية الأقل للبويضات المضمحلة عندما نحصل على البويضات من الأرانب الكبيرة في العمر إنضاجها في بيئة (TCM-199) مضاف إليها سيرم عند التلقيح أو هرموناتِ (10 IU/ml eCG, 20 IU/ml hCG).