

EFFECTS OF DIFFERENT DURATION OF CO-INCUBATION OF GAMETES ON THE DEVELOPMENT OF SHEEP EMBRYOS

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ABSTRACT. The stage of *in vitro* fertilization (IVF) is considered as a pivotal term in the embryo production programs in mammals, although the effect of this stage is still underestimated, especially in sheep. In IVF laboratories, oocytes are exposed to spermatozoa for 18 to 24 h. This prolonged duration of co-incubation for gamete can produce high levels of reactive oxygen species (ROS), which can potentially cause an increase in polyspermic penetration and hardening of the zona pellucida (ZP), which may affect embryo viability. Despite this, most researchers still prefer 18-24 h of fertilizing time for *in vitro* ewe oocytes. This study aimed to improve the success rate of the IVF process in sheep by manipulating the time of the IVF stage. In addition, penetration time of ram spermatozoa into the oocytes was measured *in vitro* condition. For this purpose, matured oocytes were incubated with capacitated spermatozoa at a concentration of 10×10^6 spermatozoa/ml in BO medium for either 6 h (Group 1, n = 119), 12 h (Group 2, n = 112), or 18 h (Group 3, n = 107). Our study showed that exposing gametes to IVF for only 6 h results in a higher fertilization rate and an improvement in the quality of the embryo development when compared to 12 h and 18-h co-incubation of gametes. Moreover, this study showed that sperm can arrive at the surface of zona pellucida already within 110 min after the co-incubation beginning and then, fertilization happens within 4.5 h after having started IVF procedure. Concluded that using the protocol adopted in this study, the highest percentage of zygote development to the blastocyst stage occurred in the shortest IVF period. In conclusion, the highest percentage of zygote development into the blastocysts stage occurs in the shortest period of IVF (6 h).

Keywords: sheep, sperm, oocytes, co-incubation of gametes, male pronucleus (MPN).

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Introduction

Millions of sperm are released inside the female reproductive tract at ejaculation, while only a few hundred contact the oocyte (Harper, 1982). On the other hand, the number of sperm that are in close proximity to the COCs (cumulus oocytes complexes) is greater during *in vitro* fertilization than it is during *in vivo* fertilization, which typically involves sperm doses that are higher than 17×10^6 spermatozoa/ml, which results in a sperm: oocyte ratio that is greater than 10,000:1 (Shi et al., 1990). Standard IVF procedures expose oocytes to sperm for 16 to 22 hours (Jafarpour et al., 2017; Mardenli et al., 2020).

Polyspermia is the most common problem in IVF (Maalouf et al., 2009; Batista et al., 2016). Long-time incubation of oocytes with spermatozoa in a high concentration can produce high levels of reactive oxygen species (ROS), which can reduce membrane fluidity by peroxidizing polyunsaturated fatty acids, causing ZP hardening and having an impact on IVF rate and embryonic development (Han et al., 2001; Lushchak, 2014; Anzalone et al., 2021). In addition, high levels of ROS increase the chance of TP53 transcriptional response, which causes cell cycle arrest and apoptosis (Pan et al., 2018). Moreover, extended gamete co-incubation time during IVF does increase polyspermia (Sumantri et al., 1997).

IVF media have traditionally been prepared to foster a favourable environment for capacitating

spermatozoa to fertilize the oocytes. However, the IVF media can produce some ROS after a few hours of incubation (Martín et al., 2008). Therefore, some fertilization media may not be ideal for incubating gametes for an extended *in vitro* fertilization time (Nedambale et al., 2006).

Although reducing the amount of time that gametes are allowed to co-incubate has been proposed as an alternative method for increasing the success rate of IVF, the results that have been obtained in various laboratories are contradictory in terms of penetration rate, monospermy, and the success rate of *in vitro* embryo development (Dirnfeld et al., 1999; Xiong et al., 2011; Liu et al., 2014; Le Bras et al., 2017; Anzalone et al., 2020). In humans, ultrashort (30 s) co-incubation can result in good fertilization rates and a low incidence of polyspermy (Bungum et al., 2006). Further, it has been shown that the length of time a gamete is incubated does not affect the development of a bovine embryo (Ward et al., 2002). These results, which were obtained with different IVF protocols, are not consistent, and they show that whether or not a shorter insemination time for oocytes will increase the success rate of *in vitro* fertilization (IVF) is still debatable.

This study aimed to determine the time needed for the sperm penetration to the oocyte and formation the male pronucleus (MPN) inside the oocyte cytoplasm during the fertilization of oocytes *in vitro*. In addition, the aim was to estimate the extent to which the development of embryos is affected by shortening the time of IVF.

Materials and Methods

Study design. The first and second groups co-incubated sperm and oocytes in 500 µL of *in vitro* fertilization media (BO) for 6 h and 12 h (shortening the incubation time). In contrast, oocytes and sperm were co-incubated in 500 µL of B0 medium for 18 h in the third group, which was part of the conventional incubation time.

***In vitro* embryo production.** IVEP was performed at the Reproductive Biology and Animal Physiology Laboratory, Ankara University, Turkey. All chemicals were acquired from the Sigma-Aldrich Chemical Company. Substances obtained from other companies are given in the relevant section.

The biological material (ovaries and fresh testes) from various Turkish breeds submitted to a local slaughterhouse was collected with AUZ1 medium and transported to the laboratory at 4°C. In the laboratory, the follicles with a diameter of 3-5 mm were aspirated using an 18 G needle connected to a 10 mL syringe.

The oocytes have at least two complete cumulus cell layers, and homogeneous cytoplasm was selected and washed twice in the oocyte collection medium (OCM). Then, 40 to 60 oocytes were placed in 500 µL IVM media TCM-199 (M199-A) supplemented with 10% FBS (16A), 1 IU/mL FSH (Life Technologies), 1 IU/mL LH (Bio98), 750 µM glutamax (35050-06), 0.36 mM sodium pyruvate (P4562), and 5 µl/ml gentamycin (GEN-10B), during 24 h at 38.5°C, 5% CO₂, and high humidity in a four-well MultiDish (Nunc™ Thermo Fisher Scientific S.L., Madrid, Spain).

Post-IVM, expanded COCs (eCOCs) were washed twice in the BO medium and placed in 500 µL (40-60) of BO medium supplemented with 5 µl/ml gentamycin, 1.25 mM sodium pyruvate, 5.00 mg/ml BSA (A3311), 1 µl/ml heparin (P4562), 40 µl/ml PHE, 2 mM caffeine and 10% FBS. Then the eCOCs were fertilized with sperm concentrations of 10×10⁶/ml. The oocytes and sperm were co-incubated at 38.5°C, with 5% CO₂ in the high humidity.

Before beginning the IVC stage, cumulus cells were partially removed using 1 µl hyaluronidase (H3884), followed mechanically by pipetting. Then, zygotes were cultured in 500 µL SOF medium supplemented with 4 g/ml BSA, 10 µl/ml glutamax, 10 µl/ml sodium citrate, 10 µl/ml Non-Essential AA (M7145), 20 µl/ml Essential AA (B6766), 10 µl/ml myo-inositol (P4562), 0.5 µl/ml Na pyruvate, and 5 µl/ml gentamycin (GEN-10B). Zygotes were incubated at 38.5°C, 5% CO₂, and 90% humidity. Every 24 h, embryonic development was monitored.

Nuclear evaluation of oocytes and embryos. Staining with 2.5 mg/ml Hoechst 3342 in 3:1 (vol/vol) glycerol/PBS and mounting on microscope slides with coverslips, sealed with nail polish, and storing at 4°C in the dark. The slides were examined with UV light (200-400 nm) and an inverted microscope by LEICA (model number: LEICA DM IL LED; Wetzlar, Germany).

Statistical analysis. Data were analyzed by one-way ANOVA using SPSS version 23.0 statistical software, and the results are presented as the mean (± SEM). Duncan's multiple range test was used to

compare differences between treatments, and P-value < 0.05 was considered significant.

Results and discussion

The efficiency of IVF was assessed using three different co-incubation times (6 h, 12 h, and 18 h) during IVF (table 1). In the 6 h incubation time, the highest fertilization rate (79.3%) occurred ($P < 0.05$), which constitutes a significant difference ($P < 0.05$) as compared with 18 h incubation time (71.4%). There were no significant differences between 6 h and 12 h incubation times, as the fertilization rate for 12 h was 74.7%. 80.7% of fertilized oocytes reached the cleavage stage in the 6 h incubation treatment, which represents superior significance ($P < 0.05$) along with other treatments as shown in table 2, which also clarifies how there were no significant differences between 12 h and 18 h in the cleavage rate of embryos, with 73.2% and 70.1%, respectively.

Table 1. Effect of sperm–oocyte co-incubation time on fertilization rate, %

Co-incubation time, hour	No. of oocytes*	No. of fertilized oocytes	Fertilization rate, %
6 ^a	150	119	79.3±1.5 ^a
12 ^{ab}	150	112	74.7±2.4 ^{ab}
18 ^b	150	107	71.4±1.7 ^b

Notes: * data from 5 replicates; mean values in the same column with different superscripts differ at $P < 0.05$.

At the morula stage, the 6 h treatment had a superior effect ($P < 0.05$) where it was 64.7%, whereas there were no significant differences between 12 h and 18 h in the morula rate, with 56.2% and 55.1%, respectively. In addition, this was no different with the rate of blastocysts, where it was the highest ($P < 0.05$) at 6 h treatment (42.0%), followed by the other treatments at 12 h and 18 h, 31.2% and 26.2%, respectively. For *in vitro* fertilization, there is still controversy about how long gametes should be co-incubated. We are interested in determining if manipulating the gamete co-incubation times is sufficient to lower the occurrence of polyspermic fertilization and analyse its effect on embryonic development rates.

Table 2. Effect of sperm–oocyte co-incubation time on embryonic development rate, %

Co-incubation time, hour	No. of fertilized oocytes*	Cleavage rate (%)	Morula rate (%)	Blastocysts rate (%)
6	119	80.7±2.0 ^a	64.7±0.9 ^a	42.0±0.6 ^a
12	112	73.2±1.3 ^b	56.2±1.8 ^b	31.2±0.5 ^b
18	107	70.1±1.2 ^b	55.1±2.7 ^b	26.2±0.8 ^c

Notes: * data from 5 replicates; mean values in the same column with different superscripts differ at $P < 0.05$.

Our data showed that increasing the co-incubation time for oocytes that had been fertilized for more than 6 h led to a greater likelihood of abnormal fertilization and a worse rate of embryonic development. In contrast, when the co-incubation duration lasted 6 h, there was a high rate of sperm penetration and zygote cleavage. The cleavage rates at 12 h of incubation were the same as those at 18 h.

These explanations may be why the cleavage and morula rates at 6 h of incubation in this study have a higher level; oocytes are typically exposed to sperm for a period of 18–26 h during the IVF procedure performed on small ruminants (Zabihi et al., 2019; Serra et al., 2021; Mousavi et al., 2022). Furthermore, the number of sperm reaching the oocytes *in vivo* is lower than the number present during IVF (Umehara et al., 2018). If there are too many spermatozoa for a long time, they can produce ROS (De Lamirande, Gagnon, 1995); this can reduce membrane fluidity by peroxidizing polyunsaturated fatty acids (Mailloux, 2020), cause the ZP to harden (Gualtieri et al., 2021), and change the quality of the oocytes (Leite et al., 2017).

ROS production rises when *in vitro* circumstances include 5% CO₂ in the incubation; therefore, as time rises, a greater quantity of these products will be created (Agarwal et al., 2022). These factors can

potentially affect even the epigenetics of the embryos (Canovas et al., 2017). In addition, centrifugation can cause harm to sperm by causing the peroxidation of spermatozoa membranes and the production of high levels of ROS, both of which can have a detrimental impact on the fusion of sperm and oocytes as the amount of time rises in IVF (Watson, 2000; Canovas et al., 2017).

Moreover, in contrast to the circumstances that prevail *in vivo*, a significant prevalence of polyspermy is observed after *in vitro* fertilization; this indicates that *in vitro* conditions are less than ideal (Alcântara et al., 2020; Nguyen et al., 2021).

Polyspermic penetration has been shown to occur during *in vitro* fertilization in various animal species. However, this phenomenon appears exceptionally high in sheep and pigs (Coy, Avilés, 2010; Alcântara et al., 2020). These data suggest that oocytes might fare better if incubated for a shorter time. Consequently, the hypothesis has been put forward that decreasing the amount of time expended in co-incubation for gametes can improve the effectiveness of the *in vitro* embryo production procedure.

Previous studies confirmed our data, which show that in humans, an incubation time of 16 h can have a negative impact on the outcomes of IVF procedures due to the excessive production of ROS and other harmful byproducts of sperm metabolism in the IVF medium (Dirnfeld et al., 1999; Xiong et al., 2011; Liu et al., 2014). It has been shown that allowing gametes and oocytes to co-incubated for less time during IVF increases the fertilization of oocytes and the formation of blastocysts (Le Bras et al., 2017; Anzalone et al., 2020). In contrast to these results, a previous study (Chen et al., 2019) found that the amount of time that gametes were co-incubated together did not affect zygote cleavage or embryonic development post IVF with unsorted ram sperm. In contrast to the case with sheep, the incubation for 10 h had no negative impact on the quality of the bovine zygote during IVF (Ward et al., 2002).

In addition, evaluations of pronuclear formation and zygote development were carried out after IVF and 72 h of IVC (Fig. 1, 2). We found that sperm can reach the zona pellucida 110 min post the co-incubation for gametes begins and that fertilization happens within 4.5 h of the start of IVF.

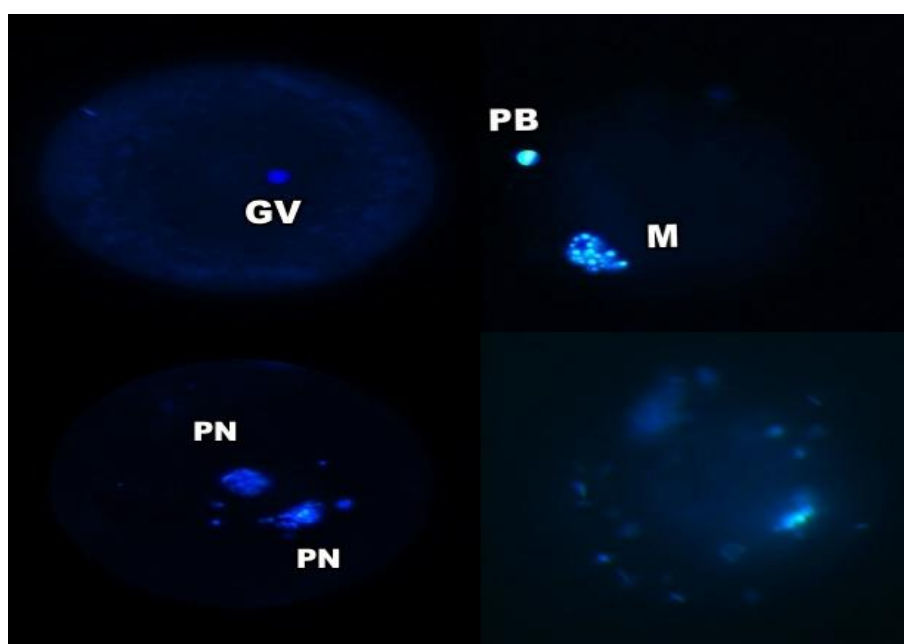


Figure 1. Fluorescence microscopy images (400x) of ewe oocytes and zygote stained with Hoechst 33342.

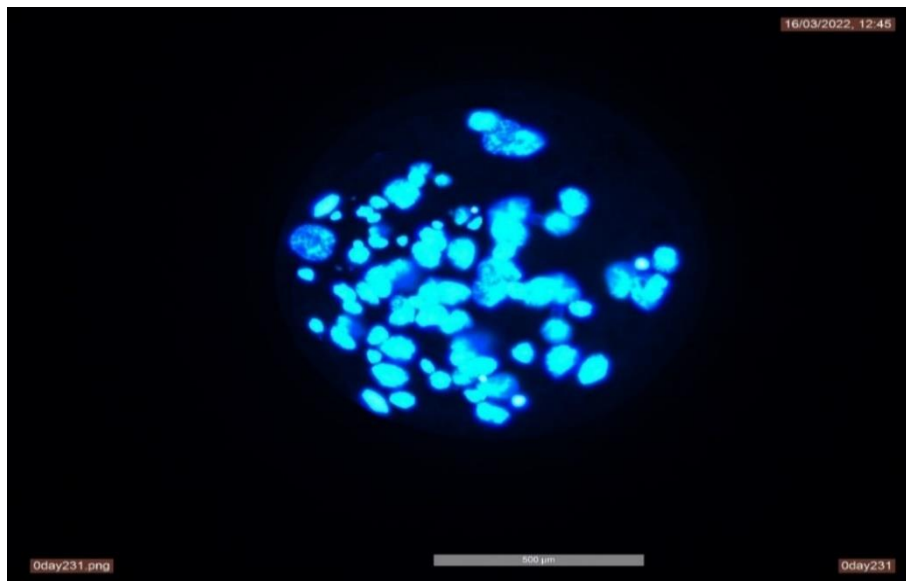


Figure 2. Fluorescence imaging of blastocysts stained with Hoechst 33342.

There were detected effects of gamete co-incubation length on the speed and rates of blastocyst formation. It is well known that *in vitro* embryonic development speed and viability/quality have a well-documented link (Lee et al., 2012). These results are in line with a study in which human IVF-embryos formed from a shorter time of gamete co-incubation had a higher implantation and pregnancy rate than those derived from a long time of gamete co-incubation (Kattera, Chen, 2003).

Conclusions

This study reveals that 4.5 h of interaction between sperm and oocytes is adequate to accomplish fertilization, to lower the incidence of polyspermy, and improve the rate at which embryos progress to the blastocyst stage without compromising the quality of the embryos.

The obtained data give grounds to conclude that using the protocol adopted in this study there is no need for exposure up to 6 hours to remove the fertilized oocytes from the IVF medium to ensure the stability of the sperm nucleus within the cytoplasm of the oocyte and not to expose them to collateral damage (temperature, pH, light, and stress induced by pipetting) during their transfer to the culture media for embryonic development.

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References

1. Agarwal A., Maldonado Rosas I., Anagnostopoulou C., Cannarella R., Boitrelle F., Munoz L.V, Finelli R., Durairajanayagam D., Henkel R., Saleh R.. Oxidative stress and assisted reproduction: a comprehensive review of its pathophysiological role and strategies for optimizing embryo culture environment. *Antioxidants*. 2022, 11(3): 477-499. DOI: <https://doi.org/10.3390/antiox11030477>
2. Alcântara-Neto A.S., Fernandez-Rufete M., Corbin E., Tsikis G., Uzbekov R., Garanina A.S., Coy P., Almiñana C., Mermillod P. Oviduct fluid extracellular vesicles regulate polyspermy during porcine *in vitro* fertilisation. *Reprod., Fertil. Develop.* 2020, 32(4): 409-418. DOI: <https://doi.org/10.1071/RD19058>
3. Anzalone D., Czernik M., Palazzese L., Ressaissi Y., Scapolo P., Loi P. 119 Short spermatozoa-oocyte co-incubation improves outcomes of IVF in sheep. *Reprod., Fertil. Develop.* 2020, 32(2): 186-195. DOI: <https://doi.org/10.1071/RDv32n2Ab119>

4. Anzalone D.A., Palazzese L., Czernik M., Sabatucci A., Valbonetti L., Capra E., Loi P. Controlled spermatozoa–oocyte interaction improves embryo quality in sheep. *Sci. Rep.* 2021, 11(1): 1-9. DOI: <https://doi.org/10.1038/s41598-021-02000-z>
5. Batista R.I., Moro L.N., Corbin E., Alminana C., Souza-Fabjan J.M., de Figueirêdo F.V.J., Mermillod P. Combination of oviduct fluid and heparin to improve monospermic zygotes production during porcine in vitro fertilization. *Theriogenology*. 2016, 86(2): 495-502. DOI: <https://doi.org/10.1016/j.theriogenology.2016.01.031>
6. Bungum M., Bungum L., Humaidan P. A prospective study, using sibling oocytes, examining the effect of 30 seconds versus 90 minutes gamete co-incubation in IVF. *Human Reproduction*. 2006, 21(2): 518-523. DOI: <https://doi.org/10.1093/humrep/dei350>
7. Canovas S., Ross P.J., Kelsey G., Coy P. DNA methylation in embryo development: epigenetic impact of ART (assisted reproductive technologies). *Bioessays*. 2017, 39(11): 1-11. DOI: <https://doi.org/10.1002/bies.201700106>
8. Chen Z.Q., Wang Y., Ng E.H., Zhao M., Pan J.P., Wu H.X., Teng X.M. A randomized triple blind controlled trial comparing the live birth rate of IVF following brief incubation versus standard incubation of gametes. *Hum. Reprod.* 2019, 34(1): 100-108. DOI: <https://doi.org/10.1093/humrep/dey333>
9. Coy P., Avilés M. What controls polyspermy in mammals, the oviduct or the oocyte? *Biol. Rev.* 2010, 85(3): 593-605. DOI: <https://doi.org/10.1111/j.1469-185X.2009.00117.x>
10. De Lamirande E., Gagnon C. Impact of reactive oxygen species on spermatozoa: a balancing act between beneficial and detrimental effects. *Hum. Reprod.* 1995, 10(1): 15-21. DOI: https://doi.org/10.1093/humrep/10.suppl_1.15
11. Dirnfeld M., Bider D., Koifman M., Calderon I., Abramovici H. Shortened exposure of oocytes to spermatozoa improves in-vitro fertilization outcome: a prospective, randomized, controlled study. *Hum. Reprod.* 1999, 14(10): 2562-2564. DOI: <https://doi.org/10.1093/humrep/14.10.2562>
12. Gualtieri R., Kalthur G., Barbato V., Longobardi S., Di Rella F., Adiga S.K., Talevi R. Sperm oxidative stress during in vitro manipulation and its effects on sperm function and embryo development. *Antioxidants*. 2021, 10(7): 1025-1049. DOI: <https://doi.org/10.3390/antiox10071025>
13. Han K.S., Kang H.J., Kim E.Y., Yoon W.J., Sohn S., Kwon H.J., Gwag B.J. 1, 2-bis (2-Aminophenoxy) ethane-N, N, N', N'-tetraacetic acid induces caspase-mediated apoptosis and reactive oxygen species-mediated necrosis in cultured cortical neurons. *J. Neuroch.* 2001, 78(2): 230-239. DOI: <https://doi.org/10.1046/j.1471-4159.2001.00394.x>
14. Harper M.J.K. Sperm and egg transport. In: *Reproduction in mammals: Germ cells and fertilization*. (Austin C.R., Short R.V, eds). Cambridge: Cambridge University Press. 1982. P. 102-127. DOI: <https://cir.nii.ac.jp/crid/1570291225119435904>
15. Jafarpour F., Hosseini S.M., Ostadhosseini S., Abbasi H., Dalman A., Nasr-Esfahani M.H. Comparative dynamics of 5-methylcytosine reprogramming and TET family expression during preimplantation mammalian development in mouse and sheep. *Theriogenology*. 2017, 89: 86-96. DOI: <https://doi.org/10.1016/j.theriogenology.2016.10.010>
16. Kattera S., Chen C. Short coincubation of gametes in in vitro fertilization improves implantation and pregnancy rates: a prospective, randomized, controlled study. *Fert. Steril.* 2003, 80(4): 1017-1021. DOI: [https://doi.org/10.1016/S0015-0282\(03\)01154-3](https://doi.org/10.1016/S0015-0282(03)01154-3)
17. Le Bras A., Hesters L., Gallot V., Tallet C., Tachdjian G., Frydman N. Shortening gametes co-incubation time improves live birth rate for couples with a history of fragmented embryos. *Syst. Biol. Reprod. Med.* 2017, 63(5): 331-337. DOI: <https://doi.org/10.1080/19396368.2017.1336581>
18. Lee M.J., Lee R.K., Lin M.H., Hwu Y.M. Cleavage speed and implantation potential of early-cleavage embryos in IVF or ICSI cycles. *J. Ass. Reprod. Genet.* 2012, 29(8): 745-50. DOI: <https://doi.org/10.1007/s10815-012-9777-z>
19. Leite R.F., Annes K., Ispada J., De Lima C.B., Dos Santos É.C., Fontes P.K., Nogueira M.F., Milazzotto M.P. Oxidative stress alters the profile of transcription factors related to early development on in vitro produced embryos. *Oxid. Med. Cell. Long.* 2017, 13: 1-14. DOI: <https://doi.org/10.1155/2017/1502489>
20. Liu W., Liu J., Zhang X., Han W., Xiong S., Huang G. Short co-incubation of gametes combined with early rescue ICSI: an optimal strategy for complete fertilization failure after IVF. *Hum. Fert.* 2014, 17(1): 50-55. DOI: <https://doi.org/10.3109/14647273.2013.859746>
21. Lushchak V.I. Free radicals, reactive oxygen species, oxidative stress and its classification. *Chem.-Biol. Interact.* 2014, C224: 164-175. DOI: <https://doi.org/10.1016/j.cbi.2014.10.016>
22. Maalouf W.E., Lee J.H., Campbell K.H. Effects of caffeine, cumulus cell removal and aging on polyspermy and embryo development on in vitro matured and fertilized ovine oocytes. *Theriogenology*. 2009, 71(7): 1083-1092. DOI: <https://doi.org/10.1016/j.theriogenology.2008.12.001>
23. Mailloux R.J. An update on mitochondrial reactive oxygen species production. *Antioxidants*. 2020, 9(6): 472-485. DOI: <https://doi.org/10.3390/antiox9060472>
24. Mardenli O., Mohammad M.S., Alolo A.Y. Effect of combination of follicle size, FSH and cysteamine on in vitro production of sheep embryos. *J. Ilmu Ternak Veter.* 2020, 25(3): 131-138. DOI:

- <http://dx.doi.org/10.14334/jitv.v25i3.2517>
25. Martín-Romero F.J., Miguel-Lasobras E.M., Domínguez-Arroyo J.A., González-Carrera E, Álvarez I.S. Contribution of culture media to oxidative stress and its effect on human oocytes. *Reproductive biomedicine online*. 2008, 17(5): 652-61. DOI: [https://doi.org/10.1016/S1472-6483\(10\)60312-4](https://doi.org/10.1016/S1472-6483(10)60312-4)
 26. Mousavi F.S., Ahmadi E., Shirazi A., Shams-Esfandabadi N., Nazari H. The effect of chemical treatment of the sheep embryo zona pellucida on the ability of blastocysts to hatch after vitrification and warming. *Veter. Med. Sci*. 2022, 8(1): 405-410. DOI: <https://doi.org/10.1002/vms3.632>
 27. Nedambale T.L., Du F., Xu J., Chaubal S.A., Dinnyes A., Groen W., Faber D., Dobrinsky J.R., Yang X., Tian X.C. Prolonging bovine sperm–oocyte incubation in modified medium 199 improves embryo development rate and the viability of vitrified blastocysts. *Theriogenology*. 2006, 66(8): 1951-1960. DOI: <https://doi.org/10.1016/j.theriogenology.2006.04.044>
 28. Nguyen H.T., Dang-Nguyen T.Q., Somfai T., Men N.T., Beck Woerner B., Viet Linh N., Xuan Nguyen B., Noguchi J., Kaneko H., Kikuchi K. Excess polyspermy reduces the ability of porcine oocytes to promote male pronuclear formation after in vitro fertilization. *Anim. Sci. J.* 2021, 92(1): e13650. DOI: <https://doi.org/10.1111/asj.13650>
 29. Pan B., Yang H., Wu Z., Qazi I.H., Liu G., Han H., Zhou G. Melatonin improves parthenogenetic development of vitrified–warmed mouse oocytes potentially by promoting G1/S cell cycle progression. *Int. J. Mol. Sci.* 2018, 19(12): 4029-4045. DOI: <https://doi.org/10.3390/ijms19124029>
 30. Serra E., Gadau S.D., Leoni G.G., Naitana S., Succu S. Seasonal effect on developmental competence, oxidative status and tubulin assessment of prepubertal ovine oocyte. *Animals*. 2021, 11(7): 1886-1898. DOI: <https://doi.org/10.3390/ani11071886>
 31. Shi D.S. Effects of bulls on fertilization of bovine oocytes and their subsequent development *in vitro*. *Theriogenology*. 1990, 30: 324-333. DOI: [available at]
 32. Sumantri C., Boediono A., Ooe M., Murakami M., Saha S., Suzuki T. The effect of sperm-oocyte incubation time on in vitro embryo development using sperm from a tetraparental chimeric bull. *Anim. Reprod. Sci.* 1997, 48(2-4): 187-195. DOI: [https://doi.org/10.1016/S0378-4320\(97\)00052-3](https://doi.org/10.1016/S0378-4320(97)00052-3)
 33. Umehara T., Kawai T., Goto M., Richards J.S., Shimada M. Creatine enhances the duration of sperm capacitation: a novel factor for improving in vitro fertilization with small numbers of sperm. *Hum. Reprod.* 2018, 33(6):1117-1129. DOI: <https://doi.org/10.1093/humrep/dey081>
 34. Ward F., Enright B., Rizos D., Boland M., Lonergan P. Optimization of in vitro bovine embryo production: effect of duration of maturation, length of gamete co-incubation, sperm concentration and sire. *Theriogenology*. 2002, 57(8): 2105-2117. DOI: [https://doi.org/10.1016/S0093-691X\(02\)00696-9](https://doi.org/10.1016/S0093-691X(02)00696-9)
 35. Watson P.F. The causes of reduced fertility with cryopreserved semen. *Animal reproduction science*. 2000, 60: 481-492. DOI: [https://doi.org/10.1016/S0378-4320\(00\)00099-3](https://doi.org/10.1016/S0378-4320(00)00099-3)
 36. Xiong S., Han W., Liu J.X., Zhang X.D., Liu W.W., Liu H., Huang G.N. Effects of cumulus cells removal after 6 h co-incubation of gametes on the outcomes of human IVF. *J. Assi. Reprod. Genet.* 2011, 28(12): 1205-1211. DOI: <https://doi.org/10.1007/s10815-011-9630-9>
 37. Zabihi A., Shabankareh H.K., Hajarian H., Foroutanifar S. Resveratrol addition to in vitro maturation and in vitro culture media enhances developmental competence of sheep embryos. *Dom. Anim. Endocr.* 2019, 68: 25-31. DOI: <https://doi.org/10.1016/j.domaniend.2018.12.010>

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Влияние различной продолжительности коинкубации гамет на развитие эмбрионов овец

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Стадия экстракорпорального оплодотворения (ЭКО) считается ключевым термином в программах производства эмбрионов у млекопитающих, хотя влияние этой стадии все еще недооценивается, особенно у овец. В лабораториях ЭКО ооциты подвергаются воздействию сперматозоидов от 18 до 24 часов. Эта длительная продолжительность совместной инкубации гамет может привести к высоким уровням активных форм кислорода (АФК), что потенциально может вызвать увеличение проникновения полиспермии и отверждение блестящей оболочки (ЗР), что может повлиять на жизнеспособность эмбриона. Несмотря на это, большинство исследователей по-прежнему предпочитают 18-24 часа оплодотворения ооцитов овцы *in vitro*. Данное исследование было направлено на повышение успешности получения эмбрионов *in vitro* у овец с регистрацией времени проникновения сперматозоидов барана в ооциты. Для этого созрелые ооциты инкубировали с капацированными сперматозоидами в концентрации 10×10^6 сперматозоидов/мл в среде БО в течение 6 ч (I группа, n = 119), 12 ч (II группа, n = 112), 18 ч (II группа, n = 112). или 34 ч (III группа, n = 107). Показано, что воздействие ЭКО на гаметы только в течение 6 ч приводит к более высокой частоте оплодотворения и улучшению развития эмбриона по сравнению с 12- и 18-ч совместной инкубацией гамет. Более того, это исследование показало, что сперматозоиды могут прибыть на поверхность блестящей оболочки уже через 110 мин после начала коинкубации, а затем оплодотворение происходит в течение 4,5 ч после начала процедуры ЭКО. Заключение, что при использовании принятого в данном исследовании протокола оптимальное развитие зиготы до стадии бластоцисты происходило в самый короткий период ЭКО (6 ч).

Ключевые слова: овцы, сперматозоиды, ооциты, коинкубация гамет, мужской пронуклеус (МПН).

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