Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 28 (2), 427-437
Mustafa Kemal University Journal of Agricultural Sciences, 28 (2), 427-437
https://dergipark.org.tr/tr/pub/mkutbd
e-ISSN: 2667-7733

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Effects of exposure to 2G/3G cell phone radiation on *in vitro* fertilization, subsequent development and sex distribution of bovine embryos

Sığır embriyolarında 2G/3G cep telefonu sinyallerinin *in vitro* fertilizasyon, embriyo gelişimi ve cinsiyet dağılımı üzerine etkileri

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ARTICLE INFO

Article history:
Recieved / Geliş: 15.11.2022
Accepted / Kabul: 11.04.2023

Keywords:
Electromagnetic field
Embryo
Sex ratio
IVF
Bovine

Anahtar Kelimeler:
Elektromanyetik alan
Embriyo
Cinsiyet
IVF
Sığır

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ABSTRACT

During the thousand years of human evolution, the male to female ratio was practically equal, but it has recently changed in some way. The aim of this study was to investigate the effects of electromagnetic radiation from mobile phones on *in vitro* fertilization, embryo growth, and sex differentiation in cattle embryos. MII oocytes obtained from ovaries taken from slaughterhouse were used as research material. Gametes were exposed to electromagnetic fields by having a mobile phone inside the incubator that would periodically ring. On days 7 and 8, blastocyst development stages and embryo cleavage rates were evaluated. Additionally, the rates of cleavage for different time intervals after *in vitro* fertilization were noted. The sex determination of the embryos produced *in vitro* was determined by using polymerase chain reaction (PCR). As a result, it was found that exposure to radiofrequency electromagnetic fields could mainly reduce blastomere count, embryo diameter, and embryo quality rather than a having major adverse effect on the development of cattle embryos. Additionally, it was shown that exposure to electromagnetic fields appears to drastically reduce the chances of male survival.

ÖZET

Bin yıllık insan evrimi boyunca eşit olan erkek/dişi oranı, son yıllarda bir şekilde bu dengeden uzaklaşmaktadır. Yapılan bu çalışmanın amacı, cep telefonlarından yayılan elektromanyetik radyasyonun sığır embriyolarında *in vitro* fertilizasyon, embriyo gelişimi ve cinsiyet farklılaşması üzerindeki etkilerinin araştırılması olmuştur. Kesimhaneden alınan ovaryumlardan elde edilen MII oositler deneme materyali olarak kullanılmıştır. İnkübatör içerisine yerleştirilen bir cep telefonunun belirli aralıklarla çaldırılması ile gametler elektromanyetik alana maruz bırakılmıştır. Embriyo gelişim aşamaları, bölünme ve 7-8'inci günlerdeki blastosist gelişim aşamaları bakımından takip edilmiştir. *İn vitro* koşullarda üretilen embriyolarda cinsiyet tayini polimeraz zincir reaksiyon (PCR) ile belirlenmiştir. Sonuç olarak radyofrekans elektromanyetik alanların embriyo gelişimi üzerine olumsuz bir etkisinin olmadığı, ancak radyofrekans elektromanyetik alanların blastomer sayısını, embriyo gelişim aşamasını ve embriyo kalitesini azaltabileceği belirlenmiştir. Ayrıca elektromanyetik alanlara maruz kalmanın erkek embriyoların yaşama şansını büyük ölçüde azalttığı tespit edilmiştir.

Cite/Atıf Güzey, Y.Z., & Önal, A.G. (2023). Effects of exposure to 2G/3G cell phone radiation on *in vitro* fertilization, subsequent development and sex distribution of bovine embryos. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 28* (2), 427-437. https://doi.org/10.37908/mkutbd.1205044

INTRODUCTION

Each day, living organisms are subjected to a rising number of electromagnetic fields (EMF) from a wide range of sources (Gye & Park, 2012). Because of the increasing need for electronic gadgets, the number of artificial sources of electromagnetic radiation (EMR) has increased dramatically. During the thousand years of human evolution, the male/female ratio has been almost equal, however, this ratio has shifted on occasion (Pourlis, 2009). The biological mechanisms underlying a skewed sex ratio are yet unknown (Irgens et al., 1997). However, Jauchem (2008) sorted a range of environmental factors influencing male/female offspring ratio as heterogeneity, paternal age, maternal age, birth season, geographical latitude, pollution, exposure to metal fumes and polychlorinated biphenyls, proximity to petrochemical, polymer and chemical industrial plants and acute physiological stress. Another widely accepted theory, as Irgens et al. (1997) summarized, for a skewed sex ratio in offspring is that a high level of gonadotrophin and a low level of testosterone can result in a lower male proportion in men. A high estrogen concentration in women is assumed to result in a high male proportion, whereas a high progesterone concentration favours females. According to another claim, enhanced estrogen levels as a result of suppressed melatonin secretion due to EMF can disturb the balance in favour of males. The gender of mammalian offspring is controlled by both parents' hormone levels at the time of conception in a way that high levels of gonadotrophin and progesterone are linked to the subsequent birth of females while high levels of estrogen and testosterone are related to the subsequent birth of males (James, 1995).

Many studies have been published on the adverse effects of EMF on sex hormones, reproductive performance, and sex ratio (Lotfi & Shahryar, 2010). The effects of EMF radiation on implantation and fetal development were discussed by Gye & Park (2012).

Under the influence of EMF, spermatocyte viability or fertilizing capacity may be diminished resulting in a lower male proportion. EMF exposure to spermatozoa can also emerge in the female genital tract and it is a known fact that male gametes can survive up to 6 days before conception (Irgens et al., 1997). Gye & Park (2012) attributed the decline in male proportion to EMF's negative impact on the Y-chromosome and changes in sex hormone concentrations. Furthermore, exposure to EMF may result in a reduced male proportion of offspring in both men and women (Irgens et al., 1997).

An effect of EMF on sex ratio alteration has also been suggested based on the data recently obtained from birth records (Auger et al., 2011; Baste et al., 2012; Buchner et al., 2014).

The focus of this research was to collect objective data on *in vitro* fertilization (IVF), embryo development, and sex differentiation in cattle embryos while they were exposed to electromagnetic fields from mobile phone signals.

MATERIALS and METHODS

This experiment was carried out in Hatay Mustafa Kemal University, embryo culture laboratory. The stock media used for *in vitro* embryo production (IVP) were purchased from Caisson Labs (Sugar City, ID, USA) to prepare Tyrode's Albumin Lactate Pyruvate (TALP) media as previously described by Parrish (2014) and Loureiro et al. (2007).

The oocyte collection media (OCM) was TCM-199 (tissue culture media) with Hanks' salts which was supplemented with penicillin (100 U mL^{-1}), streptomycin (100 µg mL^{-1}) and 5% fetal bovine serum (FBS, v/v). The oocyte maturation media (OMM) was TCM-199 with GlutamaxTM without HEPES (Thermo Fisher Scientific) supplemented with sodium bicarbonate (2.2 mg mL^{-1}), FBS (10% v/v), gentamycin (5 µg mL^{-1}), sodium pyruvate (0.22 mg mL^{-1}), estradiol, luteinizing hormone (LH) and follicle stimulating hormone (FSH).

In vitro meiotic maturation (IVM) of immature oocytes

The ovaries were collected from a local slaughterhouse in Hatay, Turkiye and transported in warmed saline supplemented with penicillin and streptomycin (P/S). IVP was performed as previously described (Soto et al., 2003) but in brief, the follicular fluid, including cumulus-oocyte complexes (COCs), was aspirated using a disposable syringe from follicles 2-8 mm in diameter. Classification of COCs was performed in OCM and under a stereomicroscope according to the method of Boni et al. (2002). COCs were then rinsed and transferred into untreated 4-well dishes containing OMM for maturation (Day -1). *In vitro* maturation (IVM) of oocytes took place in a humidified atmosphere of 5% CO_2 in the air and lasted for 18-22 h. COCs were then rinsed and transferred into IVF-TALP media. The swim-up method was used for the separation of the motile fraction of the frozen-thawed semen (Parrish, 2014). The sperm concentration was determined using a hemocytometer after the pellet was resuspended and diluted to ensure a final concentration of 1×10^6 spermatozoa mL^{-1}. The fertilization procedure was completed by adding diluted sperm, 5 µg mL^{-1} heparin and PHE cocktail (20 µM penicillamine, 10 µM hypotaurine, 1 µM epinephrine in final concentration) solution into fertilization media containing oocytes. IVF lasted for 8-12 h under conditions of humidified atmosphere and 5% CO_2 in the air. Putative zygotes were denuded of cumulus cells by gentle vortexing in HEPES-TALP supplemented with 1000 U mL^{-1} hyaluronidase and transferred randomly into culture media.

In vitro culture of embryos was performed under a humidified atmosphere of 5% CO_2 and 5% O_2 with a balance of N_2. Cleavage rates were monitored 6 times (at 26, 28, 30, 32, 34, and 36th hours) and final rates were assessed on Day 3 (Day 0 regarded as the day of *in vitro* fertilization). Blastocyst formation, morphology and quality parameters were assessed on Days 7 and 8. Embryos were stained for 10 min in 1 µg mL^{-1} Hoechst 33342 according to the method of Moreira et al. (2001) and the number of cells for each embryo was counted using fluorescent images of Hoechst-stained nuclei. Blastocyst-stage embryos were scored according to Gardner et al. (2000) considering morphology.

Exposure of gametes to EMF

A mobile phone (Motorola® Talkabout T180) was placed next to the culture petri inside the incubator working at 1800 MHz band range and generating 1.22 W kg^{-1} on the ear and 0.39 W kg^{-1} in the body SAR values (factory setting) has been used to apply EMF on gametes during fertilization. Continuous missed calls were performed for 5 min/2 h during the fertilization process to obtain data on the effect of EMF on oocyte fertilization.

Sex determination of IVP embryos

The determination of embryo sex was performed according to the procedure described by Hendricks et al. (2010). Here in brief, blastocysts were removed from the culture drops on Day 7, washed in 1 mg polyvinylpyrrolidone (PVP) in 1 mL phosphate-buffered solution (PBS-PVP) and transferred into 0.1% (w/v) protease from *Streptomyces griseus* in PBS (pronase) to remove any cumulus cells or sperm. Embryos were then collected individually in 0.1% (w/v) diethylpyrocarbonate in water (DEPC) after washing in PBS-PVP collected individually in DEPC H_2O and stored at –20°C until analysis.

Approximately 1 million somatic cells were centrifuged at 600 G for 5 minutes, the supernatant was removed and centrifuged again at 600 G following the addition of 1 mL of DEPC water to ensure a positive control. The DEPC water was removed carefully to leave the pellet and was resuspended in 200 µL of DEPC water. Aliquots were stored at -20°C until analysis.

The technique for sexing using gDNA as a template was performed as described previously (Park et al., 2001) with minor modifications. Briefly, embryos were thawed at room temperature and centrifuged at 2000 G for 5 secs, heated to 98°C for 10 min and centrifuged at 2000 G for 5 secs before the addition of PCR reagents.

Following the thawing of the DNA from the embryo, the components for the first PCR amplification were added into the tubes to ensure a final volume of 22 µL. The male and female DNA and dH_2O without DNA were used as the positive and negative control, respectively. The reaction mixture included 10× PCR buffer, 2.5 mM dNTPs, 50 mM $MgCl_2$, 1 unit of Taq DNA polymerase (Thermo Scientific, USA) and 2 µM Y-specific forward primer (5'-GATCACTATACATACACCACT-3') and 2 µM Y-specific reverse primer (5'-GCTATGCTAACACAAATTCTG-3'). After the first 10 cycles, tubes were centrifuged at 2000× G for 5 secs before the addition of the second PCR mix for autosomal primers containing 2 µM forward (5'-TGGAAGCAAAGAACCCCGCT-3') and 2 µM reverse primers (5'-TCGTCAGAAACCGCACACTG-3') for additional 23 cycles. PCR primers used to determine embryo sex were (1) Y-chromosome-specific primers that amplify a 141 base pair (bp) product and (2) autosomal bovine-specific satellite sequence primers that amplify a 216 bp product. The PCR amplifications were carried out using MultiGene Thermal Cycler (Labnet International, USA) which programmed for an initial denaturation at 95°C for 7 min followed by 10 cycles of 95°C for 30 secs, 55°C for 30 secs and 72°C for 30 secs. After 33 cycles, the reaction mixtures were kept at 72°C for 7 min. PCR amplification products were separated by electrophoresis on 3% (w/v) agarose gel (Sigma-Aldrich Co Ltd., Poole, UK) in a 1× TBE buffer (89.0 mM Tris, 88.9 mM boric acid, 2.2 mM EDTA, pH 8.3) containing 1 µg ml^{-1} ethidium bromide. The gels run at 140 V for 80 min and photos were taken under UV light using the DNR MiniLumi (DNR Bio-Imaging Systems, Israel) gel documentation system. If only one band of the bovine-specific product was visible on the gel, the blastomere was considered to be derived horn female embryo while the presence of two bands indicated a male embryo.

Statistical analysis

Data were collected in 7 replicates for the experiment. The IBM-SPSS (v23 for Windows) program was used for all statistical analyses. Percentage data were tested for the normal distribution using the UNIVARIATE procedure option NORMAL. Data were arc-sin transformed (arc-sin(root square)) when necessary since they did not have a normal distribution.

The percentage of the total number of cleaved oocytes and developmental stages (early, mid, expanding and expanded blastocyst) was subjected to ANOVA (GLM procedure) using a model that included the fixed effect of the electromagnetic field to compare differences between groups.

To assess the changes over developmental stages (2-cell, 8-cell, early and mid-blastocyst, expanding and expanded blastocyst and blastocyst yield on Day 8) of cleaved oocytes, REPEATED MEASURES analyses were conducted using a model with SPSS. The model-based mean±standard error (SEM) is reported for each outcome at the developmental stage. Using a Bonferroni adjustment for the comparison of the developmental stages, these mixed model analyses were also used to identify significance within mean differences between follow-up points for each outcome (Bonferroni adjusted α=0.05). The relationships between the electromagnetic field and embryo development were examined by these covariates in the mixed models.

Embryonic cell counts and embryo diameters were analyzed using independent samples t-test. Embryo quality scores were evaluated utilizing Mann-Whitney U and the sex distribution of embryos was analyzed using the chi-square test. The data were presented as mean±SE. The p-value used to determine the significance in all tests was 0.05.

RESULTS

The number of successfully sexed embryos developed to the blastocyst stage was 208 out of 325 in the present study. Developmental stages of the embryos for cleavage and blastocyst on Days 7 and 8 were evaluated.

Even though the electromagnetic field exposure resulted in a modest increase and a two-hour delay in the cleavage of cattle embryos (Figure 1), no significant differences in the cleavage and 8-cell at 48 hours following insemination were observed (Table 2).

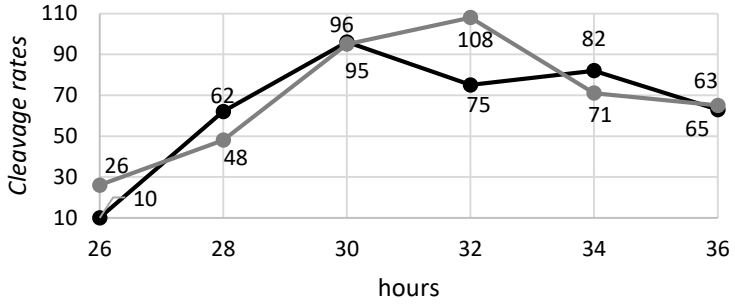

Figure 1. Cleavage rates for every 2 hours from 26h to 36h following insemination (n)
Şekil 1. Tohumlamadan (n) sonraki 26 saatten 36 saate kadar her 2 saatte bir bölünme oranları

A total of 388 putative zygotes cleaved and developed to the 2-cell stage, of which 251 embryos were arrested at further developmental stages and only 137 (35.3%) embryos progressed and developed to the blastocyst stage in the control group, whereas the same ratio was 188 of 413 putative zygotes (46%) in exposure group embryos (Tables 1 and 2). Cleavage rates for 26, 28, 30, 32, 34 and 36 hours following the *in vitro* fertilization were recorded. Cleavage rates only for 26 h post insemination were significantly higher ($p = 0.05$) for the exposure group than that in the control. Furthermore, the cleavage rate of the embryos continued to rise at a peak at 32 h under the effect of EMF and 30 h for the control group following insemination (Table 1).

Table 1. Cleavage timing of the putative zygotes determined in the study
Çizelge 1. Çalışmada belirlenen aday zigotların bölünme zamanlaması

Hours	Cleavage		P	8-cell		P
	C (n=388)	EMF (n=413)		C (n=130)	EMF (n=164)	
26	2.23±0.08 (10)	5.80±0.08 (26)	0.05	80.00±1.68 (8)	69.23±0.93 (18)	0.078
28	13.81±0.18 (62)	10.71±0.25 (48)	NS	53.23±0.78 (33)	64.58±0.73 (31)	NS
30	21.38±0.18 (96)	21.21±0.33 (95)	NS	42.71±0.58 (41)	50.53±0.66 (48)	NS
32	16.70±0.34 (75)	24.11±0.19 (108)	NS	29.33±1.15 (22)	42.59±0.53 (46)	NS
34	18.26±0.35 (82)	15.85±0.33 (71)	NS	25.61±1.30 (21)	21.13±0.42 (15)	NS
36	14.03±0.21 (63)	14.51±0.05 (65)	NS	7.94±0.34 (5)	9.23±0.31 (6)	NS

Values are % ± SEM (n). C: control, EMF: electromagnetic field, NS: not statistically significant

The blastocyst rate of the embryos on Day 7 and Day 8 was significantly higher for the exposure group ($p < 0.05$) than those in the control group (Table 2).

Table 2. Developmental stages of the embryos determined in the study
Çizelge 2. Çalışmada belirlenen embriyoların gelişim evreleri

Developmental stage	Control	EMF	P-value
2-cell	86.4±0.14 (388)	92.2±0.11 (413)	0.076
8-cell at 48h	33.5±0.47 (130)	39.7±0.38 (164)	NS
Blastocyst on day 7	13.9±0.26 (54)	20.8±0.01 (86)	<0.05
Blastocyst on day 8	35.3±0.83 (137)	45.5±0.93 (188)	<0.05

Values are %±SEM (n). EMF: electromagnetic field, NS: not statistically significant

Developmental stages of the cattle embryos following morula are presented in Table 3. It was observed that the electromagnetic field exposure resulted in a significant rise in early and mid-blastocyst yields ($p = 0.02$) but did not affect the expanding/expanded blastocyst yields on Day 8. The effects of the EMF exposure and timing of cleavage were compared on 2-cell cattle embryos and their subsequent development into blastocysts (Table 3). The percentage of EMF exposed embryos that developed into blastocysts was significantly higher than that in the control group ($p < 0.05$). In the present study, it was found that the timing of cleavage significantly affected blastocyst yield at any sub-stage ($p < 0.05$).

Table 3. Developmental stages of the embryos on Day 8 based on the first cleavage time
Çizelge 3. İlk bölünme zamanına göre 8. Günde embriyoların gelişim aşamaları

Developmental stages		Hours (h)						P-value	
		26	***28***	***30***	***32***	***34***	***36***	***T***	***H***
Early&Mid Blastocyst	*C*	0.0 (0)	19.4±1.76 (12)	12.5±1.41 (12)	10.7±0.97 (8)	2.4±1.04 (2)	6.4±1.63 (4)	0.020	0.020
	EMF	11.5±2.56 (3)	25.0±1.63 (12)	23.2±1.22 (22)	12.0±1.17 (13)	9.9±1.31 (7)	7.7±1.18 (5)		
Expanding Blastocyst	*C*	10.0±2.32 (1)	14.5±1.29 (9)	15.6±0.87 (15)	16.0±1.63 (12)	8.5±1.11 (7)	1.6±0.67 (1)	NS	0.030
	EMF	19.2±1.92 (5)	10.4±1.17 (5)	16.8±1.27 (16)	16.7±1.04 (18)	14.1±1.67 (10)	3.1±0.79 (2)		
Expanded Blastocyst	*C*	0.0 (0)	25.8±1.38 (16)	22.9±1.51 (22)	14.7±1.13 (11)	6.1±1.04 (5)	0.0 (0)	NS	0.000
	EMF	19.2±1.92 (5)	18.8±1.50 (9)	23.2±1.17 (22)	25.0±1.60 (27)	9.9±1.62 (7)	0.0 (0)		
Blastocyst on day 8	*C*	10.0±2.32 (1)	59.7±1.64 (37)	51.0±1.34 (49)	41.3±1.58 (31)	17.1±1.62 (14)	7.9±1.62 (5)	0.012	0.000
	EMF	50.0±2.81 (13)	54.2±1.75 (26)	63.2±1.29 (60)	53.7±2.01 (58)	33.8±2.50 (24)	10.8±1.07 (7)		

Values are %±SEM (n). T: treatment, H: hour, C: control, EMF: electromagnetic field

Moreover, the survival rate to the blastocyst stage does not guarantee that the embryos are normal. As proof of this claim, we found that the blastomere numbers and diameters of the EMF-exposed embryos were slightly decreased. Embryo quality scores on Day 8 were higher for the control group in line with these data. None of these parameters mentioned above was statistically significant (Table 4).

Table 4. Embryo quality parameters observed in the study
Çizelge 4. Çalışmada gözlenen embriyo kalite parametreleri

	Control	EMF	P
Blastomere number	92.4±4.98	87.62±26.48	NS
Diameter (mm)	189.5±1.83	185.2±1.54	0.075
Score	1.9±0.02	1.5±0.02	NS

As a consequence of agarose gel electrophoresis in the present study, those exhibiting only one autosomal amplicon were classified as females while those exhibiting two amplicons were classified as males (Figure 2). The sex distribution of the embryos of both groups differed significantly from the expected distribution of 50:50. The sex ratio of the embryos was in favour of males in both groups but the EMF resulted in a significant ($p < 0.05$) shift in female embryos (Figure 3). The proportion of females to males was found to be 37:63 in EMF exposed group whereas it was 25:75 in the control group.

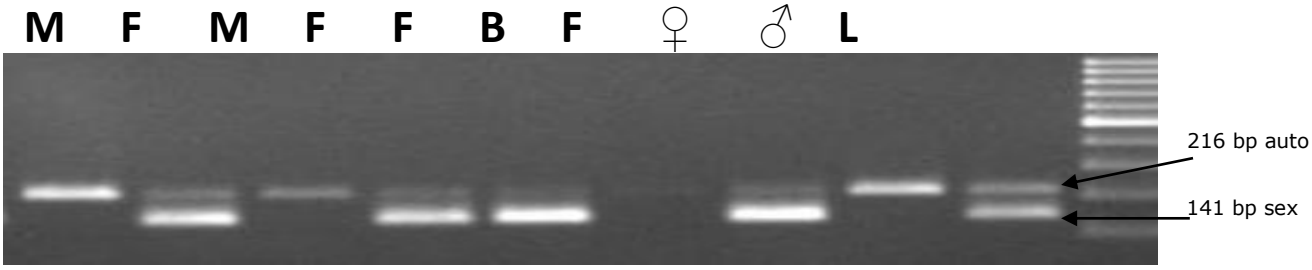

Figure 2. Representative results for the analysis of embryo sex by PCR. (L: DNA ladder; F: female; M: male, B: Blank – PCR reaction mixture without embryo. The amplicons for the Y-specific primer (141 bp) and autosomal primer (216 bp) are indicated by arrows. Note that embryos that produced both the 216 and 141 bp amplicons were classified as male while those with only the 216 bp product were classified as female).

Şekil 2. Embriyo cinsiyetinin PCR ile analizi için temsili sonuçlar. (L: DNA marker; F: dişi; M: erkek, B: Boş – embriyosuz PCR reaksiyon karışımı. Y'ye özgü primer (141 bp) ve otozomal primer (216 bp) için amplikonlar oklarla gösterilmiştir. hem 216 hem de 141 bp amplikonları üreten embriyolar erkek olarak sınıflandırılırken, yalnızca 216 bp ürünü olanlar dişi olarak sınıflandırıldı).

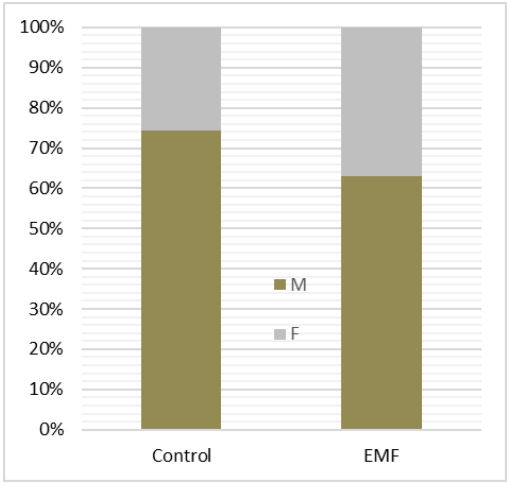

Figure 1. Effect of electromagnetic fields on the sex distribution of the embryos produced *in vitro*. χ2 test showed a significant ($p<0.05$) relationship between EMF exposure and sex distribution (F: female, M: male)

Şekil 3. Elektromanyetik alanların in vitro üretilen embriyoların cinsiyet dağılımına etkisi. χ2 testi, EMF maruziyeti ile cinsiyet dağılımı (K: kadın, E: erkek) arasında anlamlı (p<0,05) bir ilişki göstermiştir.

DISCUSSION

Early embryos at various developmental stages, such as MII oocytes and zygotes, can be used for a variety of manipulations involving electric and electromagnetic fields but little is known about the possible species-specific differences in sensitivity to such fields in early embryos at different developmental stages. The evidence on whether EMF can promote or suppress embryo cleavage is generally contradictory.

EMF exposure may cause altered proliferation of cells. Previous reports on an increase in proliferation (Katsir & Parola, 1998; Velizarov et al., 1999), cell cycle progression (Antonopoulos et al., 1995), cell colony growth (Wei et al., 2008; West et al., 1994) and an improving effect on cell survival (Grant et al., 1994) of various cell types are already available. Due to increasing intracellular Ca^{2+}, extremely low-frequency EMF increases cell proliferation, oocyte activation, and development in a dose-dependent manner (Wolf et al., 2005). Differences in the ability of individual sperm to stimulate calcium transients may result in alterations in the timing of the first zygotic cleavage (Lechniak et al., 2008). Although we did not evaluate intracellular ion concentrations, the enhanced cleavage rate could be due to the EMF's modulating role in Ca^{2+} channel regulation.

Several studies have demonstrated that embryo cleavage (EC) is a strong predictor of embryo viability but the precise time of the cleavage for each developmental stage of cattle embryos has yet to be determined (Lee et al., 2012; Stensen et al., 2010). In cattle, the period to the first cleavage ranges from 22 to 48 hours with a peak of 2-cell stage embryos around 36 hours (Lechniak et al., 2008). Early cleaved embryos have a higher rate of blastocyst formation and pregnancy rates if transferred. Because the timing of the first mitotic division is thought to be an indicator of embryo quality, the timing of cleavage can be utilized to predict embryo outcome. The data from previous studies suggest that as early embryonic development progresses, cleavage timing becomes less consistent (Arav et al., 2008; Lechniak et al., 2008). This theory was supported in the present study that 50 per cent of the 2-cell embryos cleaved within 2 h (30 to 32 h) and about 60 per cent of these embryos progressed to the blastocyst stage accounting for 63 per cent of the total embryo yield in the exposure group. On the other hand, a more uniform distribution was observed for the timing of cleavage in the control group. The proportion of the embryos cleaved at the same time interval was 44% and 47% of these embryos developed to the blastocyst stage.

In general, the electromagnetic field resulted in a delay in EC but did not affect the blastomere number, diameter and embryo quality score as reported by Roychoudhury et al. (2009). In the current study, significant differences were found in blastocyst yield but not in EC, as expected. The dose-dependency of embryo exposure is one of the factors that could explain this phenomenon. The heterogeneity of the exposure systems, various electromagnetic stimulation parameters, poor dosimetry and the unpredictability of animal models are often responsible for such conflicting results (Piccinetti et al., 2018). Furthermore, the finest quality embryos arise from early cleavage embryos (30 h) and the developmental potential declines as the time to the first cleavage increases. Embryos derived from zygotes that cleaved late (> 36 h) rarely reached the blastocyst stage. Even if a late cleaved embryo progresses into a blastocyst, the quality of the embryo is compromised and it is not selected for a routine transfer (Lechniak et al., 2008). The findings in the current study suggest that even a slight delay in the timing of the first cleavage significantly influenced embryo competence to develop into a blastocyst and the timing of blastocyst formation.

It is well documented that media supplements used in the *in vitro* culture of cattle embryos may enhance male survival and provide male embryos with a better chance to grow faster and reach more advanced developmental stages than female embryos (Gutiérrez-Adán et al., 2001; Lechniak et al., 2008). EMF appears to reduce the chances of male survival based on sex alterations and blastocyst rates. This decline in male proportion could be due to alterations in sex hormone concentrations and the detrimental effect of EMF on the Y-sex chromosome (Irgens et al., 1997; Lotfi & Shahryar, 2010). Since the reported data produced conflicting results, it is still unclear whether the majority of the embryos exposed to EMFs are females.

As a consequence, radiofrequency EMF exposure did not have a major adverse effect on the development of the cattle embryos but could mainly decrease blastomere count, embryo diameter and embryo quality regarding these findings. It was also found that EMF exposure seems to give significantly less opportunity for male survival.

STATEMENT OF CONFLICT OF INTEREST

The author(s) declare no conflict of interest for this study.

AUTHOR'S CONTRIBUTIONS

All authors have contributed equally to the experiment.

STATEMENT OF ETHICS CONSENT

Embryos are not included in the list of organisms that require specific authorization as specified in the Animal Protection Act article 2c of Directive 2011/28914, hence no approval was requested.

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