

# Effects of static magnetic fields on chick embryo mesonephros development

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## SUMMARY

Exposure to magnetic fields has been reported to affect several cellular processes, including growth and differentiation. In this study, we investigated how continuous exposure to 18 or 36 mT static magnetic fields affects the development of the chick embryo mesonephros. A total of 120 fertile White Leghorn eggs were used: 40 eggs were incubated under control conditions (incubator with the coil off) and 80 were incubated under the effects of static magnetic fields (incubator with the coil on). The embryos of the exposed groups were subjected to static magnetic fields continuously from day 0 of incubation to the moment of sacrifice. Half of the embryos in each group (both control and exposed eggs) were sacrificed on the 5th day of incubation and the other half on the 10th day. At the earlier stage of embryogenesis (5th day), the mesonephros was smaller and there were fewer mesonephric tubules in the groups exposed to static magnetic fields than in controls, suggesting that magnetic fields have an inhibitory effect on embryonic development. However, after 10 days of exposure to static magnetic fields, the stage of development was more advanced and the mesonephros was larger in the exposed groups than in the controls. Moreover, the mesonephric tubules had started to degenerate in the exposed embryo groups but not in the control group. The results suggest that static magnetic fields stimulate the development and maturity of chick embryo structures.

**Key Words:** Mesonephric tubules - Mesonephros - Magnetic fields - Chick embryo - Kidney development.

## INTRODUCTION

The mesonephros is the main excretory organ during embryonic life. The first rudiment of the mesonephros appears after 48 hours of incubation and after 72 hours it is fully developed (Wischnitzer, 1980). According to Romanoff (1962), the mesonephros is visible after 62 hours of incubation; it extends from somite 20 to somite 30 and there are between 5 and 6 tubules per somite. The mesonephros reaches its maximum stage of development after 10 days (Lillie, 1952; Romanoff, 1962). In the chick embryo, the mesonephric structures arise on the 2<sup>nd</sup> day of incubation between the 13th or 14th somite and the 30th somite and develop caudally (Friebova-Zemanova and Goncharevskaya, 1982). In the first stages, the mesonephros is differentiated by the formation of mesonephric tubules. It can be divided into three parts: the renal corpuscle, the secreting tubule (both derived from the nephrogenous tissue), and the collecting tubule, derived from evagination of the mesonephric duct. On the 5th day, tubules stop forming and a period of growth starts. At first, secreting tubules are fairly convoluted and this convolution increases over the following days (Lillie, 1952; Friebova-Zemanova, 1981). A wide sinusoidal vascular network occupies the space between the tubules and there is very little connective tissue (Lillie, 1952; Romanoff, 1962).

The mesonephric duct is located on the dorso-lateral edge of the mesonephros and the collecting tubules open into its lumen.

The chick mesonephros can be considered to be functional between the 4th and the 11th day of incubation. After this period, signs of degeneration can be observed: there is a reduction in the tubular lumen, picnosis, a loss of cellular

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Submitted: July 5, 1999

Accepted: September 10, 1999

adherence, and an increase in connective tissue (Gheri et al., 1990).

In this study, the effects of continuous exposure to static magnetic fields (SMF) on the development of the chick embryo mesonephros were analysed morphometrically on the 5th and 10th day of incubation.

## MATERIALS AND METHODS

Two identical incubators (Masalles, model 25, Spain) equipped with a temperature selector, thermostat, potentiometer, hygrometer and automatic egg turnover were used. The SMF exposure system used here has been described previously (Piera et al., 1992; Espinar et al., 1997). Briefly, SMFs were created by a solenoid coil, consisting of a rigid PVC support and a monofilament, inside each incubator. The centre of the coil was a hollow cylinder 110 mm in diameter and 160 mm in length. The solenoid was connected to a power supply (Promax FAC-5528, Spain) outside the incubators and a SMF was obtained (frequency = 0; wavelength = 0). The intensity of the SMF can be controlled between 0 and 40 mT.

Two incubators were used, both of which were identically equipped. The coil of the first incubator was not connected to the power supply. The eggs incubated in this way were the control group. The coil of the second incubator was connected to the power supply. The eggs incubated in this way were the group series exposed to a SMF. A total of 120 White-Leghorn eggs were used in eight series, each with 5 control embryos and 10 exposed embryos. Four series were exposed at 18 mT and the other four at 36 mT. In sum, 40 eggs were incubated under control conditions and 80 under the effects of SMF. The embryos of the exposed groups were subjected to SMF from the first day of incubation to the moment of sacrifice. Half of the embryos in each group (both control and exposed) were sacrificed on the 5th day and the other half on the 10th day.

All incubation conditions were controlled before, during and after each experiment. The eggs were incubated in the darkness and turned over automatically once every hour. Temperature ( $37.5 \pm 0.5^\circ\text{C}$ ) was checked with a temperature sensor placed at the centre of the egg support. The hygrometer inside the incubator revealed humidity to be above 70% all the time. The SMF, measured with a gaussmeter (Oxford Instruments, model kGauss-031T, Spain) in the laboratory and inside the incubator with the SMF system off, was 0.044 mT. All these parameters remained constant along the study.

Fertile White Leghorn eggs were purchased from a commercial hatchery (Nueva Comarcal,

Reus, Spain). Before incubation, the eggs were stored horizontally for 24h at  $4^\circ\text{C}$  with the small end of the egg facing the earth's magnetic south. The eggs were placed horizontally in a specially designed amagnetic support with their pointed end facing the magnetic south of the SMF generated. This support was introduced into the coil, which was fixed to the automatic turning device of the incubator.

The eggs from the lay were randomly assigned by computer to a dose group, a sacrifice day, and a type of study (morphometric or ultrastructural). The groups were not identified until the statistical study.

The weight, length and HH stage (Hamburger and Hamilton, 1951) were determined in all of the embryos extracted.

A morphometric study was carried out on 100 embryos. Of these, 32 were control embryos, 34 had been exposed to an SMF of 18 mT and 34 to an SMF of 36 mT. The remaining 20 embryos were used for ultrastructural studies (data not shown here).

## LIGHT MICROSCOPY AND MORPHOMETRIC STUDY

### *Light microscopy*

The embryos were processed with the conventional method. They were fixed in 10% buffered formalin solution, embedded in paraffin, and cut in  $7 \mu\text{m}$  serial sections (sagittally or transversally). Sections were stained with hematoxylin-eosin. Ten-day-old embryos were decalcified by chloral hydrate.

*Selecting a section:* After we had observed all of the serial sections of one embryo, we selected one of them for the morphometric determinations (diameters and number of tubular intersections). The choice depended on the plane in which the section had been made. The sagittal section was taken where the cranio-caudal distance of the mesonephric duct was greatest, and the transverse section at the mid-point, where an equal number of cranial and caudal sections of the mesonephros could be observed on either side. (Fig. 1).

### *Morphometry*

Mesonephric diameters and volume, and the number of tubular interceptions were determined.

A semiautomatic analysis system (MOP-Videoplan 2000, Kontron, Germany) was used. For diameter measurements, a micrometer rule was placed at the centre of the mesonephros, and the diameters were measured as the distances between the ends of the mesonephros on the horizontal and the vertical axes. Transverse and sagittal diameters were determined in transverse sections, while sagittal and vertical diameters

were determined in sagittal sections (Fig 1). The 3<sup>rd</sup> diameter for each mesonephros was estimated as:

$$\text{Diameter} = N \times T$$

where N is the number of mesonephros serial sections; T, the thickness of the sections (7µm), and the estimated diameter was the transverse (sagittal sections) or the vertical one (transverse section).

The volume (Vol) of each mesonephros was calculated by assuming the structure to be cylindrical on the 5th day and prismatic on the 10th day. The mathematical formulae applied were:

$$\text{Vol}_5 = \pi \phi V \times \frac{(\phi S + \phi T)^2}{4}$$

$$\text{Vol}_{10} = \phi V \times \phi S \times \phi T$$

where, Vol<sub>5</sub> and Vol<sub>10</sub> are the volume in mm<sup>3</sup> on the 5th and 10th day, and φV, φS, and φT are the vertical, sagittal and transverse diameters.

### STATISTICAL ANALYSIS

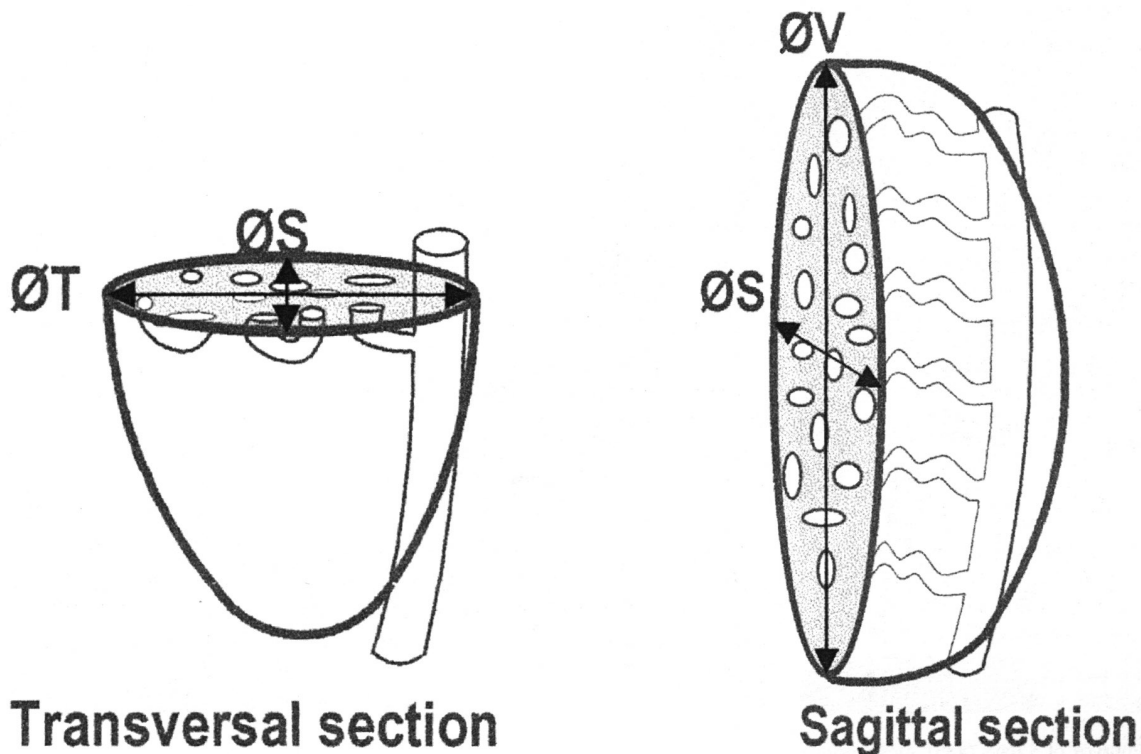
All parameters were measured in a double blind trial by a single observer. The diameters and the number of tubular interceptions were determined in both the right and left mesonephros of each embryo and the arithmetic mean of the right and left ones was calculated. The results are expressed as the mean and standard deviation. Non-parametric procedures were used (Kruskal-Wallis and the Mann-Whitney tests) for multiple comparisons to detect statistical differences among the three groups (SPSS statistical package, SPSS Inc., Chicago, Illinois). The distribution of qualitative variables (HH-stage) was compared using the Pearson χ<sup>2</sup> test. The significance level was fixed at p<0.01 in each test.

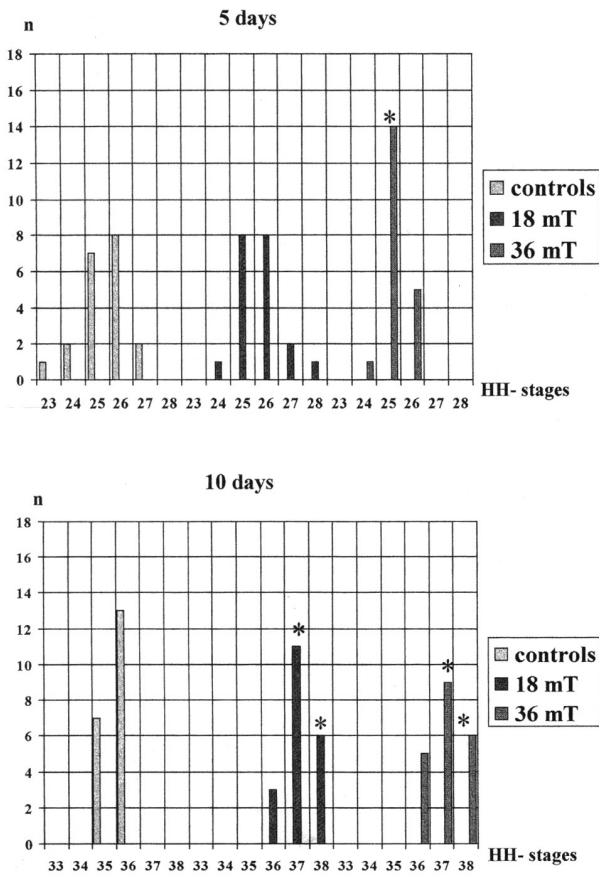
### RESULTS

#### WEIGHT, CROWN-RUMP LENGTH AND HH-STAGE

The lengths and weights of the 5- and 10-day-old embryos in the exposed groups were not statistically different from the respective lengths

**Fig. 1.** Schemes of the mesonephros: φV = vertical diameter; φT = transverse diameter; φS = sagittal diameter; MD = mesonephric duct; MT = mesonephric tubules.





**Fig. 2.** Histogram of embryological stages (HH-stage). \*  $p < 0.01$  vs control group (Pearson  $\chi^2$  test). n = number of embryos.

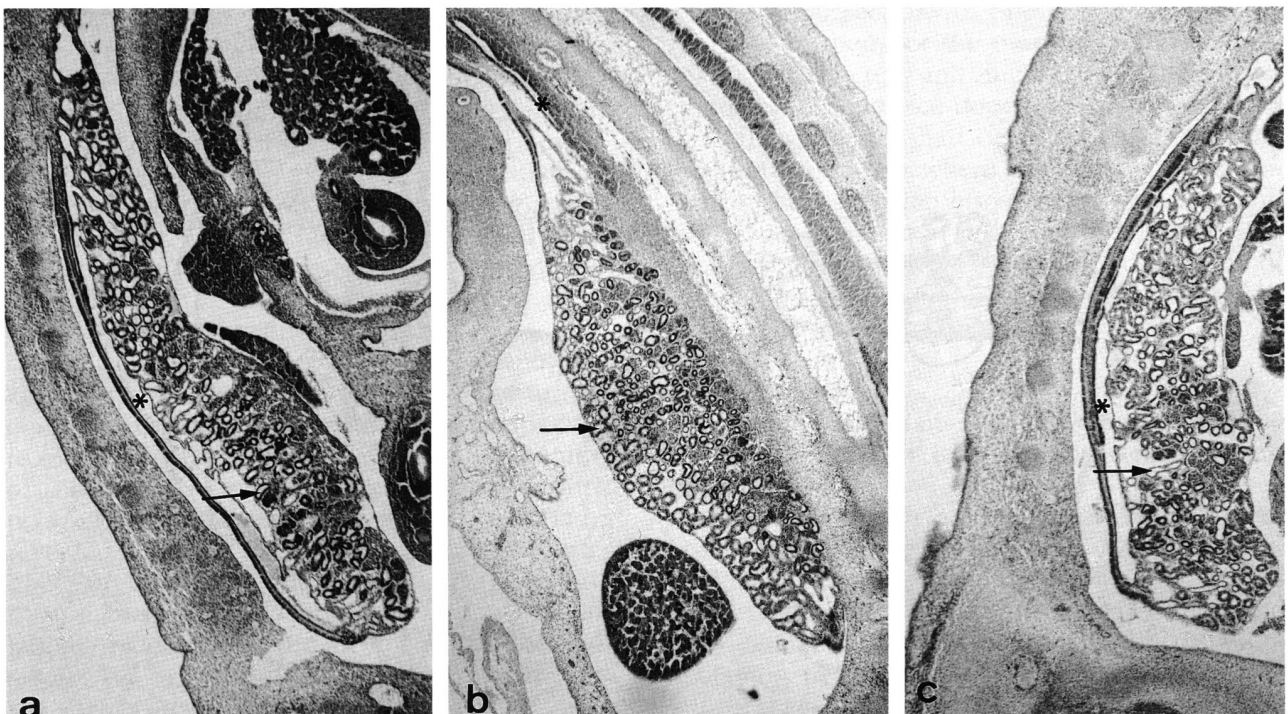
and weights in the control groups (Table 1). Figure 2 shows the HH-stage of five- and ten-day old embryos. On the 5th day, the stages of

the embryos exposed to 18 mT varied considerably but this was also the case in the controls (between stages 23-28). A statistically higher percentage of embryos was found at a lower HH-stage (stage 25) in the group exposed to 36 mT than in controls ( $p < 0.01$ ). On the 10th day, both groups had more advanced HH-stage (stages 37 and 38) embryos than the controls ( $p < 0.01$ ).

#### DIAMETERS AND VOLUMES OF THE MESONEPHROS

The length of the transverse, sagittal, and vertical diameters of the mesonephros was statistically smaller in embryos exposed to SMF than in the controls on the 5th day of incubation ( $p < 0.01$ , Table 2) but no differences were found between the two exposed groups. On the 10th day, the transverse and vertical diameters of the mesonephros of embryos exposed to SMF were higher than in the controls ( $p < 0.01$ ). The sagittal diameter was shorter in exposed embryos than in the controls ( $p < 0.01$ , Table 2). The only differences between the two pairs of exposed groups at 10 days were in their vertical diameters. These were statistically smaller in embryos exposed to 18 mT than in those exposed to 36 mT ( $p < 0.01$ , Table 2).

The volume of the mesonephros in five-day old embryos exposed to SMF was smaller than in the controls ( $p < 0.01$ ) (Fig. 3). However, on the 10th day the volume of the mesonephros was statistically greater in both groups of exposed embryos than in the controls ( $p < 0.01$ , Table 3).



**Fig. 3.** Embryos of 5 days (x35). Mesonephros sagittal section. a = control; b = exposed to 18 mT; c = exposed to 36 mT. (\*) = mesonephric duct, (→) = mesonephric tubules.

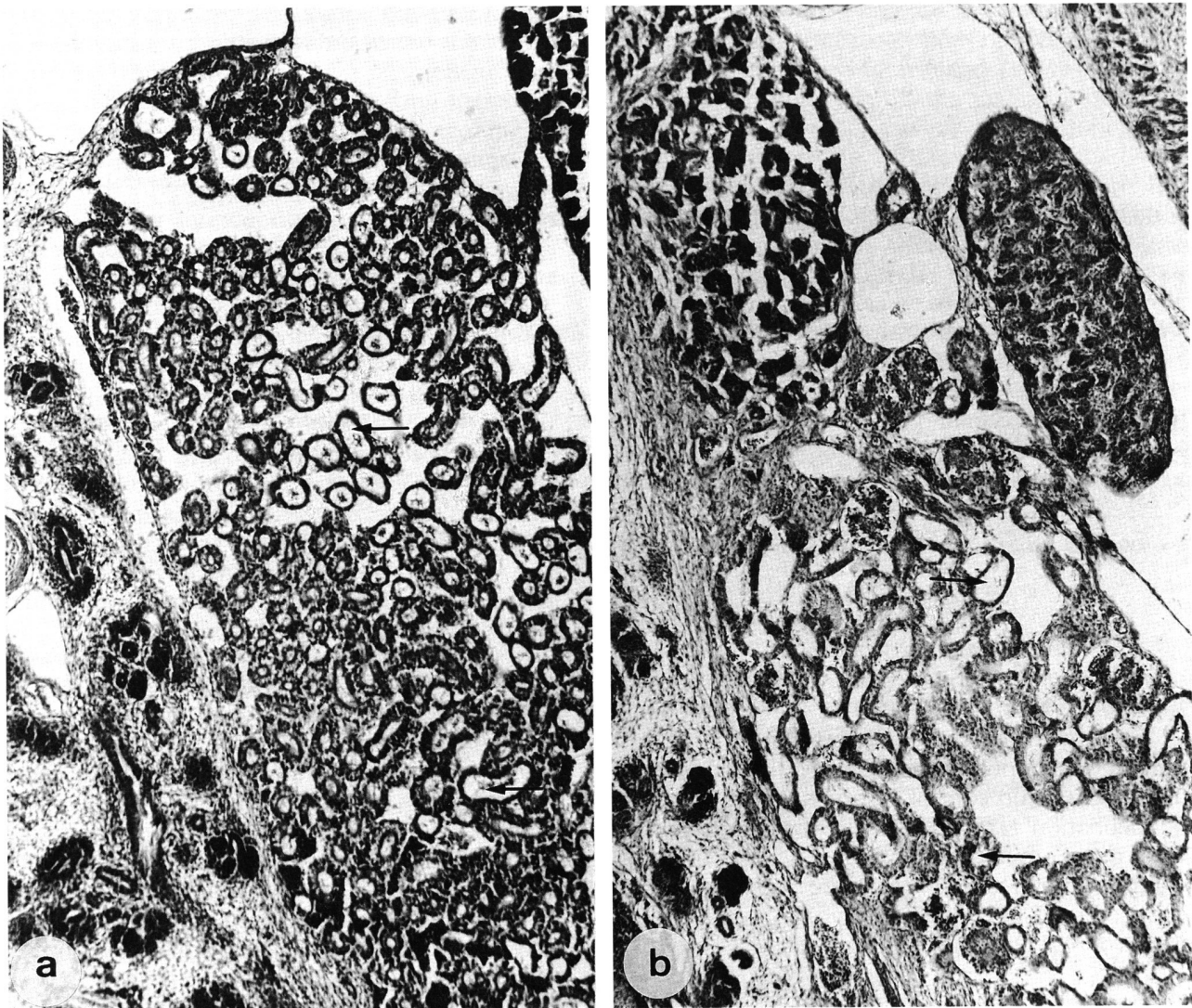


Fig. 4. Embryos of 10 days (x80). Mesonephros sagittal section. a = control; b = exposed to 36 mT. (→) = mesonephric tubules.

Table 1. Weight (g) and length (mm) of 5 and 10-day-old embryos. Mean ± SD

Group	5 days			10 days		
	n	Weight	Length	n	Weight	Length
Control	20	0.13 ± 0.03	9.9 ± 2.6	20	2.02 ± 0.38	28.6 ± 3.5
18 mT	20	0.11 ± 0.05	8.8 ± 2.7	20	1.98 ± 0.49	30.4 ± 2.8
36 mT	20	0.13 ± 0.02	8.7 ± 1.3	20	1.98 ± 0.27	26.2 ± 2.5

n = number of embryos

Table 2. Transverse, sagittal and vertical diameters (mm) of mesonephros. Mean ± SD

Diameter	5 days			10 days		
	control n = 16	18 mT exposure n = 17	36 mT exposure n = 17	control n = 16	18 mT exposure n = 17	36 mT exposure n = 17
Transverse	0.43 ± 0.03	0.32 ± 0.07*	0.30 ± 0.05*	0.90 ± 0.09	1.12 ± 0.15*	1.13 ± 0.10*
Sagittal	0.54 ± 0.04	0.34 ± 0.10*	0.35 ± 0.06*	1.78 ± 0.10	1.33 ± 0.14*	1.44 ± 0.17*
Vertical	2.79 ± 0.08	2.10 ± 0.27*	2.09 ± 0.29*	3.83 ± 0.07	4.43 ± 0.52**	4.83 ± 0.36**

n = number of embryos. \*p < 0.01 vs control (Kruskall-Wallis test) \*\*p < 0.01 (Kruskall-Wallis test)

**Table 3.** Volume (mm<sup>3</sup>) of mesonephros. Mean  $\pm$  SD

Group	5 days		10 days	
	n	Volume	n	Volume
Control	16	0.53 $\pm$ 0.08	16	6.18 $\pm$ 1.09
18 mT	17	0.17 $\pm$ 0.08*	17	6.75 $\pm$ 2.16*
36 mT	17	0.16 $\pm$ 0.06*	17	7.90 $\pm$ 1.28*

n = number of embryos. \*p < 0.01 vs control (Kruskall-Wallis test)

**Table 4.** Number of tubular interceptions in sagittal and transverse sections. Mean  $\pm$  SD

Group	5 days				10 days			
	n	Sagittal	n	Transverse	n	Sagittal	n	Transverse
Control	8	41.4 $\pm$ 22.4	8	17.1 $\pm$ 4.0	8	433 $\pm$ 29	8	119 $\pm$ 17
18 mT	11	40.2 $\pm$ 18.6	6	11.0 $\pm$ 3.9	11	268 $\pm$ 33*	6	83 $\pm$ 16
36 mT	11	41.2 $\pm$ 16.3	6	11.9 $\pm$ 4.1	11	275 $\pm$ 41*	6	121 $\pm$ 38

n = number of embryos. \*p < 0.01 vs control. (Kruskall-Wallis test)

#### TUBULAR INTERCEPTIONS

##### *Sagittal sections*

There were no differences in the number of tubular interceptions in the sagittal sections of the exposed and control embryos on the 5th day of incubation (Fig. 3). On the 10th day, there were more tubular interceptions in all groups, as compared with day 5, but there were fewer in exposed than in control embryos in sagittal sections (p < 0.01), (Table 4, Fig. 4).

##### *Transverse sections*

The differences found in the number of tubular interceptions between the exposed embryos and controls were not statistically significant at either of the ages studied (Table 4).

#### DISCUSSION

SMFs of 18 mT and 36 mT were chosen because of the average intensity of industrial and professional exposure, which is between approximately 0.3 mT and 65 mT.

Exposure to pulsed EMF has been associated with teratogenic effects in different animals (Baronoth, 1963; Joshi et al., 1978; Maffeo et al., 1984; Berman, 1990). However, SMFs of between 10 and 50mT (low intensity) have been shown to accelerate embryonic development and/or growth (Duriez and Basset, 1980; Piera et al., 1992, 1997; Espinar et al., 1997; Jové et al., 1999a,b). Other authors have described a "window effect" i.e. alterations will only be evident at certain frequencies or intensities in the biological response to magnetic fields (Adey, 1980; Ubeda et al., 1983; Blackman et al., 1985; Piera

et al., 1992; Jové et al., 1999a,b). The heterogeneity of magnetic fields, their ability to disturb some of the normal conditions of incubation (in experimental studies) and the wide variety of devices used to generate them can lead to considerable disparity in the results. Moreover, we have found no references in the literature to the relationship between SMFs and mesonephros development, making it difficult to compare our work with that of other authors. No significant differences were found in the weight and size of the exposed and control embryos. We believe that the small differences detected among the different groups of embryos may be due to the normal physiological dimorphism of all living beings. However, their external morphological characteristics (HH-stage) on incubation day 10 showed that the embryos exposed to SMFs were more advanced than the controls. The fact that these differences were more evident in the embryos exposed to intensities of 18 mT than in those exposed to 36 mT could be connected to the so-called "window-effect", the limits of which are still unknown. However, this needs to be further demonstrated in other studies with series of different intensities.

By determining the different diameters of the mesonephros and calculating its volume it is possible to establish its size and shape after 5 and 10 days of development. In the same way, these parameters can be used to analyse whether, at a particular age (5 or 10 days), there are differences between controls and exposed embryos.

The maturity of the mesonephros can be determined by the number and degree of sinuosity of the mesonephric tubules. These tubules cease to appear on day 5 of embryonic development. They are distributed in levels and each

somite has 5 to 6 tubules (Romanoff, 1962). Subsequently, the tubules develop in the transverse plane and but hardly at all in the vertical one (Lillie, 1952; Romanoff, 1962). Taking these criteria into account, the maturity of the mesonephros may be determined by counting the number of tubular interceptions. Thus, the number of tubular interceptions counted in sagittal sections can help to evaluate the number of tubules formed. The values obtained in the transverse sections give an idea of how complex and sinuous it has become since it appeared. Consequently, this can be used as an additional index of maturity.

After 5 days of incubation, the mesonephros diameters and the volume of exposed embryos were smaller than in the controls. This indicated delayed development. At the same time, the number of tubular interceptions in the sagittal sections in both groups of exposed embryos was similar to the number observed in the controls. However, there were fewer interceptions in the transverse sections of the exposed groups than in the controls. Despite the fact that the differences detected are not statistically significant, taking into account Lillie's (1952) and Romanoff's (1962) descriptions of the way in which mesonephric tubules mature, the mesonephric tubules in the embryos exposed to SMFs are less sinuous than in the controls. As a result, the mesonephros of these embryos are less mature. On the whole, after 5 days of incubation the differences detected between the embryos exposed to SMFs and the controls seem to show a less developed mesonephros.

The volume of the mesonephros on day 10 was greater than on day 5 in all groups. The volume was greater in exposed embryos than in controls, indicating advanced mesonephros development. The transverse and vertical diameters of the mesonephros of the embryos exposed to SMFs were greater than those of the controls, but the sagittal diameter was smaller. This shows that the mesonephros is getting smaller. According to Romanoff (1962), the mesonephros degenerates when the mesonephric tubules regress (on about day 11 of incubation) and this degeneration can be appreciated because the size of the lumen decreases, the cells in the wall lose their adhesion, and the basal membrane disappears.

After 10 days, there were fewer tubular interceptions in the exposed groups than in the controls. The differences detected between the exposed embryos and the controls were found in the sagittal sections but not in the transverse sections. If one applies the same criteria as after 5 days of development, these differences mean that the mesonephros is developing more quickly and that the mesonephric tubules have begun to degenerate (fewer tubules).

To sum up, the parameters studied strongly suggest that SMFs cause different and apparently contradictory effects after 5 and 10 days of development. In other words, the magnetic field can inhibit the development of the mesonephros early on in the life of the embryo (there are fewer, less sinuous tubules and therefore the volume of the mesonephros is not as great) but can then stimulate it. After 10 days, the volume of the mesonephros of the embryos exposed to 18 mT is almost the same as that of the controls whereas the volume of the mesonephros of embryos exposed to 36 mT is greater.

Further studies should be carried out on subsequent stages of development in order to confirm these findings in the metanephros, the structure which replaces the mesonephros later on in development.

## REFERENCES

- ADEY WR (1980). Frequency and power windowing in tissue interactions with weak electromagnetic fields. *Proc of the IEEE*, 68: 119-125.
- BARNOTHY MF (1963). Biological effects of magnetic fields on small mammals. *Biomed Sci Instrum*, 1: 127.
- BERMAN E (1990). The developmental effects of pulsed magnetic fields on animal embryos. *Reprod Toxicol*, 4: 45-49.
- BLACKMAN CF, BENANE SG, HOUSE DE and JOINES WT (1985). Effects of ELF (1-120 Hz) and Modulated (50 Hz) RF Fields on the Efflux of Calcium Ions From Brain Tissue In Vitro. *Bioelectromagnetics*, 6: 1-11.
- DURIEZ R and BASSETT CAL (1980). Effect of some electric signals transmitted by an induction coil on weight increase, incorporation of marker, and histological and ultrastructural appearance of the skeleton in a chick embryo. *CR Sciences Acad Sci*, 290: 1483-1486.
- ESPINAR A, PIERA V, CARMONA A and GUERRERO JM (1997). Histological changes during development of the cerebellum in the chick exposed to a static magnetic field. *Bioelectromagnetics*, 18: 36-46.
- FRIEBOVÁ-ZEMANOVÁ Z (1981). Formation of the chick mesonephros. 4. Course and architecture of the developing nephrons. *Anat Embryol*, 161: 341-354.
- FRIEBOVÁ-ZEMANOVÁ Z and GONCHAREVSKAYA OA (1982). Formation of the chick mesonephros. 5 Spatial distribution of the nephron populations. *Anat Embryol*, 165: 125-139.
- GHERI G, GHERI-BRYK SS and PETRELLI V (1990). Histochemical detection of sugar residues in the chick embryo mesonephros with lectin-horseradish peroxidase conjugates. *Histochemistry*, 95: 63-71.
- HAMBURGER A and HAMILTON HL (1951). A series of normal stages in the development of the chick embryo. *J Morphol*, 88: 49-92.
- JOSHI MV, KHAN MZ and DAMLE PS (1978). Effect of magnetic field on chick morphogenesis. *Differentiation*, 10: 39-43.
- JOVÉ M, COBOS P, TORRENTE M, GILBERT R and PIERA V (1999a). Embryonic development of pineal gland vesicles. A morphological and morphometric study in chick embryos. *Eur J Morphol*, 37: 29-35.

- JOVÉ M, TORRENTE M, GILABERT R, ESPINAR A, COBOS P and PIERA V (1999b). Effects of static magnetic fields on chick embryo pineal gland development. *Cells Tissues Organs*, 165: 74-80.
- LILLIE FR (1952). Lillie's development of the chick. An introduction to Embryology. New York: Holt, Rinehart, Winston.
- MAFFEO S, MILLER MW and CARSTENSEN EL (1984). Lack of effect of weak low frequency electromagnetic fields on chick embryogenesis. *J Anat*, 139: 613-618.
- PIERA V, RODRÍGUEZ A, COBOS A, TORRENTE M and COBOS P (1992). Influence of continuous electromagnetic fields in the stage (HH), weight and stature of the chick embryo. *Acta Anat*, 145: 302-306.
- PIERA V, ESPINAR A, JOVÉ M, TORRENTE M, COBOS P and PÉREZ-CASTILLA J (1997). Efecto de los campos electromagnéticos continuos sobre el desarrollo del tejido óseo del embrión de pollo. *Rehabilitación*, 31: 294-300.
- ROMANOFF AL (1962). The avian embryo. Structural and functional development. New York: The Macmillan Company.
- UBEDA A, LEAL J, TRILLO MA, JIMENEZ MA and DELGADO JM (1983). Pulse shape of magnetic fields influences chick embryogenesis. *J Anat*, 137: 513-536.
- WISCHNITZER S (1980). Atlas and laboratory guide for vertebrate embryology. Barcelona, Ed. Omega S.A.