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EFFECT OF CHRONIC EXPOSURE TO THE PESTICIDE SELECRON® ON OVULATION RATE, PROGESTERONE LEVEL AND CHROMOSOMAL ABNORMALITIES IN EWES

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SUMMAY

This experiment was carried out on 13 crossbred ewes divided into 2 groups. Group 1 (n=6) received no treatment and served as control. Group 2 (n=7) given an oral dose of 0.25 ml the organic phosphorus compound Selecron for 3 months. After the end of Selecron administration, both the control and experimental ewes received half norgestomet ear implant for 12 days. At implant removal, 800 IU PMSG was injected i.m. Ewes were monitored for oestrus, timing to oestrus and duration of oestrus. Ovulation rate was determined on day- 6 post oestrus using transabdominal ultrasound scanning and mid-ventral laparotomy. Blood samples were collected before norgestomet application, before PMSG injection, during oestrus and on day 6-post oestrus. Serum progesterone level was measured using RIA. Results indicated that timing to oestrus was significantly longer ($P<0.05$) and number of un-ruptured follicles was significantly greater ($P<0.05$) in Selecron treated group than control one. Number of corpora lutea (CL) was significantly greater ($P<0.01$) in control than Selecron group. Transabdominal ultrasound scanning failed to predict ovulation rate in ewes. Serum progesterone level was significantly higher ($P<0.01$) in Selecron group during oestrus, while, on day- 6 post oestrus it was significantly higher ($P<0.01$) in control than Selecron group. Cytogenetical analysis revealed that chronic exposure of ewes to Selecron pesticide significantly increase ($P<0.01$) the incidence of total aberrant cells, chromatid gaps, chromosome gaps and total structural aberrations. Also, the incidence of deletion and fragments and chromosome breaks significantly increased ($P<0.05$) af-

ter Selecron administration compared with control group. Non reciprocal translocation 1q 26-20 to 3q terminal part was found in one control ewe.

In conclusion, chronic exposure of ewes to the organic phosphorus pesticide, Selecron impair reproductive function as indicated by the significant decreased in ovulation rate, serum progesterone level and marked increase in the incidence of chromosomal abnormalities.

Key words: Selecron; ovulation rate; ultrasound; progesterone levels and chromosomal aberrations.

INTRODUCTION

Methods to improve reproduction in sheep often aim to increase the proportion of ewes having twin ovulation and thereby increase the lambs crop. The ovulation rate could be increased in ewes through genetic manipulation (Cornu and Cognie, 1984), nutrition (Boukhlig *et al.*, 1996), and immunization against polyandroalbumin (Geldard *et al.*, 1984) and by using exogenous gonadotropins (Tokos and Tokosova, 1990; Naqvi and Gulyani, 1998). Gonadotropin increased the ovulation rate in sheep by: (1) recruiting small follicles; (2) causing up to three fold increase in the follicular growth rate; (3) altering the size distribution of the largest follicles at oestrus but not by reversing artresia (Driancourt and Fry, 1992).

Nowadays, pesticides are widely spread used in agriculture for controlling insects and in animals for combating external parasites. Exposure of farm animals to pesticides has an adverse effect on their reproductive performance. In this respect, reports demonstrated that pesticides produced sterility in male by affecting spermatogonia stages (Krause and Homola, 1974). In females, the reproductive system has been reported to be a target of pesticides at all stages of development. Pesticides produces a decrease in ovarian weight and an increase in the number of large atretic follicles in rat (Martinez and Swartz, 1991). Degeneration of granulosa cells was also reported in rats (Cumming and Perreault, 1990). In bovine, organochlorine pesticides impair fertility by the direct action on oocyte meiotic progression and early embryonic development *in vitro* (Alm *et al.*, 1994; Faundez *et al.*, 1996). Furthermore, pesticides produced structural chromosomal damage in both somatic and germ cells of mouse in a dose-dependent manner (Abd El Aziz *et al.*, 1993). Meanwhile, it had been reported that chromosomal abnormalities adversely affecting the ovarian response to gonadotropin (Hassanene *et al.*, 1995 ; Mahrous and Abdoon, 1996). Selecron is the most extensively used pesticide for protecting agricultural crops in Egypt during 1997(Amer *et al.*, 2000).

Available literatures are lacking any figures concerning the effect of organic phosphorus compound, Selecron on ovulation rate in ewes.

Therefore the present work was designed to investigate the effect of chronic low dose exposure to Selecron pesticide (OPC) on ovulation rate, progesterone level and chromosomal abnormalities in ewes.

MATERIALS AND METHODS

Experimental animals:

The present work was carried out during the non-breeding season. Thirteen non-pregnant crossbred ewes (2-3 years old, 35-40 kg body weight) raised in the Experimental Farm of The National Research Center, Abou Rawash. Ewes were kept in an open housing system. Each ewe was fed on 0.75 kg commercial concentrate mixture (Protein not less than 16%). Rice straw, barseem (Trifolium alexandrium, during May-December) and water were provided ad libitum. The routine system used by the Egyptian Organization for Veterinary Service for vaccination and combating of infectious diseases was followed.

Insecticide:

Selecron® 720 Emulsion (Organic Phosphorus Compound, 72%) was chosen as it was used extensively in most of Egyptian villages during 1997 especially in Kalyoubia governorate. The active principle is Profenofos [(4bromo-2-chlorophenyl) 0-ethyle-s-propyl phosphorothioate}, produced by Ciba Giegy Ltd., Basle, Switzerland, batch No 8225 in the form of white emulsion. It is used in agricultural field as a diluted solution (75 ml Selecron: 100 liter water / fadan) for spraying of vegetables and crops once/ season.

Experimental design:

Ewes were divided into two groups. Group 1 (n=6) drenched 10 ml of distilled water and served as control. Group 2 (n=7) received daily oral dose of 0.25 ml of Selecron in 10 ml distilled water for 3 consecutive months (4.8µg / kg live body weight). The previously mentioned dose was calculated according to field bases, by considering the ingested amount of contaminated barseem by each ewe is 5 kg/day and the average yield of fadan from barseem as 3000 kg/season (Amer *et al.*, 2000). After the end of Selecron administration, ewes in both control and experimental groups received half norgestomet ear implant (Crestar®, Intervet, The Netherlands) containing 1.5 mg norgestomet. Crestar oil solution (1ml) containing 1.5 mg norgestomet and 2.5 mg oestradiol valerate was given i.m. at implant insertion. Implant was left in place for 12 days, 800 IU PMSG (Folligon®, Intervet, The Netherlands) was injected i.m. at implant removal. Ewes were monitored for oestrus, allowed to to be mated using intact rams. Percentage of responded ewes, timing to oestrus and duration of oestrus were recorded.

Ovulation rate was determined on day- 6 post oestrus using Transabdominal Real -Time ultrasound scanning and t confirmed by mid-ventral laparotomy. Laparotomy was performed under

general tranquilizer (0.1 ml Roumpon®, Bayer, Germany). Number CL and un-ruptured follicles were counted and recorded. After laparotomy, one animal in the control group did not respond to the gonadotropin and its data were excluded from results. Further, cytogenetical analysis of this animal indicated the presence of non-reciprocal translocation.

Blood sampling and progesterone assay:

Blood samples (5 ml) were collected by direct Jugular veine puncture; (1) before norgestomet application, (2) just before PMSG injection, (3) during estrus and (4) on day 6 post- oestrus. Clear serum was separated and stored at -20°C until progesterone assay. Progesterone levels were measured (Radioimmunoassay) using kits from Diagnostic Product Corporation, (Los Angeles, USA) according to Pratt et al. (1991). The sensitivity of the assay was 0.02 ng/ml. The intra and inter-assay coefficients of variation were 4.65 and 8.00%, respectively.

Ultrasonography:

Ovulation rate was determined on day- 6 post oestrus through Transabdominal Real-Time ultrasound examination using 3.5-5.0 MHz probe (Scanner 480 Vet.,Pie Medical, Mastricht, The Netherlands). The apparatus was rent from the Animal Reproduction Research Institute. Ewes were restrained for scanning while they were setting down with their hind limbs extended horizontally. Wool was removed from the lower belly area and skin was coated bilaterally with echogel. Image was freezed on monitor and printed out.

Cytogenetical analysis:

Blood samples (5 ml) were collected from the control and Selecron groups before Crestar application. Blood cells were cultured for 72 h. in 5-ml TCM-199 medium, 0.1 ml fetal calf serum and 0.1ml phytoheamagglutinine (PHA). After incubation, cells were treated with colchicine for 2 hrs, then with hypotonic solution (0.075 M Kcl) for 30 minutes at 38°C. After fixation in acetic acid: ethanol (1:3 v/v), the cell suspension was dropped on wet slides then flamed to dry, slides were stained with Giemsa stain and covered with DPX mounting media, 100 metaphase spreads were examined per animal (Halnan, 1977).

Statistical analysis:

Data were statistically analyzed using Student "t" test and Chi-square analysis according to Snedecor and Cochran (1980).

RESULTS

Data describing timing to oestrus, duration of oestrus and ovulation rate are summarized in Table (1). Data revealed that timing from PMSG injection to the onset of oestrus was significantly longer ($P<0.05$) for ewes exposed to Selecron compared to control group. Duration of oestrus was the same in both groups. Moreover, the number of CL was significantly greater ($P<0.01$)

Table (1):Effect of chronic exposure to low dose of Selecron (OPC.) on ovarian response of ewes following injection of PMSG (Mean±SE).

Group	Response of ewes(%)	Timing to estrus/hrs	Duration of estrus/hrs	Ovarian response (No) CL	un-ruptured foll
Control(n=5)	100	40.0±4.28	- 24.0	4.71±0.64	1.28±0.47
Selecron Treated (n=7)	100	56.0±4.28*	- 24.0	0.28±0.18**	4.38±0.94*

* P<0.05 ** P<0.01

Fig. (1): Ovarian response to gonadotropin in control (A) and Selecron treated (B)groups.

in control than Selecron group, whereas, the number of un-ruptured follicles was significantly greater (P<0.05) in Selecron treated group than the control one (Fig 1).

Transabdominal ultrasound scanning failed to predict the ovulation rate in both control and experimental groups. Only one case, in which two un-ruptured follicles and one CL (Fig 2) was recorded in Selecron group.

In the present work, serum progesterone levels were nearly the same in control and Selecron

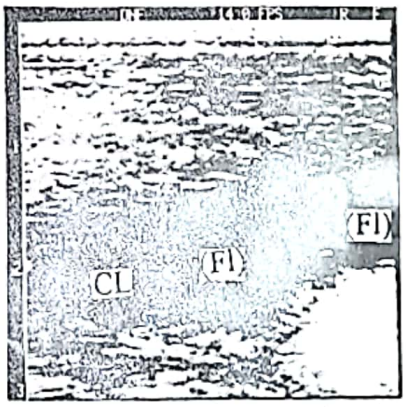

Fig. (2): Transabdominal ultrasound scanning of ovary showing two follicles (Fl) and one CL in Selecron group.

groups before Crestar application and just before PMSG injection. However, during oestrus, serum progesterone level was significantly higher (P<0.01) in Selecron group compared to control one. On day- 6 post oestrus, progesterone level reached its maximal value in the control group and being significantly higher (P<0.01) than Selecron group (Fig 3).

Cytogenetical analysis of structural aberrations and numerical variations were statistically different between Selecron and control groups (Table 2). Total aberrant cells, chromatid gaps, and chromosome gaps and total structural aberrations were significantly higher (P<0.01) in Selecron than control group. In addition, deletion and fragment and chromosome breaks (Fig.4) were Significantly increased in Selecron than control group (P<0.05). In control group, a case of chromosomal translocation (non-reciprocal translocation 1q 26-2 to 3q terminal part) was detected in

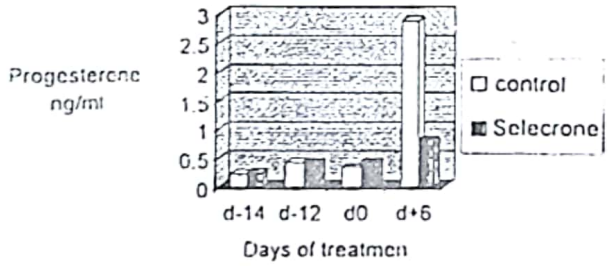

Fig. (3): Effect of chronic exposure to Selecron on serum progesterone level in ewes

Table (2): Cytogenetical analysis of blood cells in control and Selecron groups (Mean±SD).

Groups	No. examined cells	No.total aberrant cells	Gaps	Chr. Gaps	Breaks	Delection and Fragments	Chr. Breaks	Chromo Translocatin	Total structural aberration	Polyploid
Control	600	20.75± 0.83	6.75± 0.83	1.50± 0.87	6.75± 0.83	3.00± 1.00	0.00	1.25± 0.83	19.25± 0.83	1.50± 0.50
Selecron	700	38.75± 1.92**	9.25± 2.17**	6.75± 1.64**	8.50± 0.87	6.75± 2.17*	1.25± 0.83*	3.25± 0.83*	35.50± 1.66**	3.25± 0.83

*P<0.05 **P<0.01

Fig. (4): Metaphase spread showing deletion and chromatid break in Selecron treated group

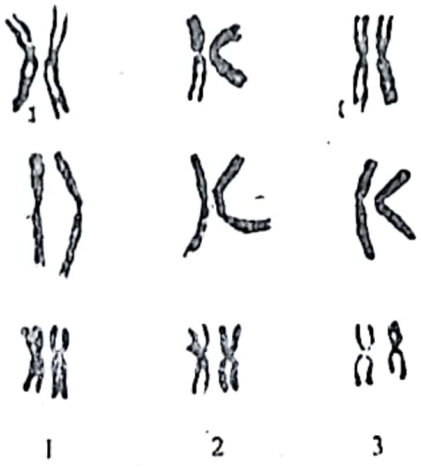

Fig. (5): Chromosome (1,3) from three partial karyotype showing the non- reciprocal translocation: 1q.26-2. to 3q terminal part.

all metaphases examined, besides the high incidence of chromatid gaps and breaks (6 and 7, respectively) in this animal (Fig 5). Data of this ewe were excluded from results, as it did not respond to the gonadotropin treatment (PMSG injection

DISCUSSION

In the present study, the effect of chronic low dose exposure to one of the most extensively used organic phosphorus compound (Selecron) used for protection of agricultural crops under the Egyptian field condition on ovulation rate, progesterone levels and chromosomal abnormalities in ewes was determined.

The obtained results showed that timing from PMSG injection to the onset of oestrus was significantly longer ($P<0.05$) in Selecron than control group. Additionally, the number of unruptured follicles on day 6-post oestrus was significantly greater ($P<0.05$) in Selecron than control group. Lopaz-Barbella *et al.* (1979) attributed the longer interval from PMSG administration to the observed estrus to the significant decrease in ovulation rate. Martinez and Swartz (1991) reported a decrease in ovarian weight and increase in the number of large atretic follicles in the ovaries of adult mice exposed to Methoxychlor (MXC). Moreover, Hexachlorocyclohexan (HCH) pesticides impair sexual receptivity, disrupted the ovarian cyclicity and reduced uterine weight in rats (Laws *et al.*, 1994). The mechanism by which pesticides can influence ovulation rate has not yet been fully understood . Bonney *et al.* (1973) postulated that exposure to pesticides results in pituitary hypofunction. Other reports suggested that pesticides (DDT, MXC and HCH) inhibited DNA synthesis, cell proliferation and steroidogensis of *in vitro* cultured granulosa cells (Tiemann *et al.*, 1996). Pesticides cause a

degeneration of granulosa cells of follicles as indicated by the atresia (Bal and Mungkornkarn, 1978). Moreover, organochlorine pesticides can influence cells responsible for reproduction by inhibiting gap junctional inter cellular communication (Tiemann and Pohland, 1999) or by altering Mag+ and ATPase activity (Tiemann and Kuchenmeister, 1999). In the present work, serum progesterone levels were nearly the same in control and Selecron groups before Crestar application and at PMSG injection. The persistent low progesterone levels in ewes during the period of implantation in the non-breeding season were expected, as ovaries are supposed to be non-functional. However, in the present study, serum progesterone level was significantly higher ($P<0.01$) in Selecron group during oestrus when compared with control. For that reason, the delayed onset of oestrus behavior may be due to a slower rate of metabolism of the synthetic progesterone used. This higher progesterone level during the preovulatory period resulted in failure of expression of oestrous behavior/ or failure of LH surge (Kafi and McGowan, 1997), and it may be, in part, responsible for the presence of a significantly higher ($P<0.05$) number of un-ruptured follicles in the present study.

In the present study, the number of CL was significantly higher ($P<0.01$) in control than Selecron treated group. High ovulation rate results from either high follicle stimulating hormone (FSH) support to follicle during the late luteal and follicular phase (McNatty *et al.*, 1985). At the same time, progeterone concentration was significantly higher ($P<0.01$) in control than Selecron treated group on day- 6 post oestrus. This higher progesterone level was parallel with the increased number of CL. Similar findings have been reported by Dinar *et al.* (1987). In addition, the low progesterone level in Selecron group may be related to the influence of exposure to the pesticide. Tiemann *et al.* (1996) found that pesticides inhibited progesterone in cultured granulosa cells *in vitro*. Amer *et al.* (2000) attributed the harmful effect of the organic phosphorus compounds to the direct cytotoxic action on gonads and pituitary especially their toxic metabolites.

In the present investigation, chronic exposure of ewes to Selecron pesticide resulted ina highly significant increase in cytogenetical abnormalities. The most prominent cytogenetical changes in exposed ewes were the significantalty higher incidence of total aberrant cells, chromatid gaps, chromosome gaps and total structural aberration. In addition, deletion and fragments and chromosome breaks were also increased significantly after Selecron exposure. These findings are close to the previous reports (Gustavsson, 1980; Weishun *et al.*, 1982). It seems that organic phosphorus compounds affecting DNA synthesis, mainly due to interference with nucleotide synthesis and consequently leading to misformation of DNA synthesis (Landolt and Koeon, 1983).

In this study a case in the control group showed chromosomal translocation and complete ovarian inactivity even after gonadotropin therapy. It was reported that females showing translocation did not responded as well to gonadotropin treatment as normal cows did (Schmutz *et al.*, 1991), and translocation was detected in a subfertile bull (Villagomez *et al.*, 1993). So, the low ovulation rate in the present study may be attributed to the higher incidence of chromosomal aberrations following exposure to Selecron.

In conclusion, chronic exposures even to a low dose of the pesticide Selecron impair reproductive function of ewes as indicated by the significant decrease in ovulation rate and low serum progesterone levels and the marked increase in the incidence of chromosomal abnormalities.

REFERENCES

Abel Aziz, K.; El Nahas, E. and Halima, S.A. (1993): Tamaron-induced sperm shape abnormalities and chromosomal aberrations in mouse. J. Egypt. Ger. Soc.Zool., 12:99-113.

Alm, H.; Tiemann, U. and Torner, H. (1994): Influence of organochlorine pesticides on maturation of bovine oocytes in vitro. J. Reprod. Fert. , 14: 66 (Abst).

Amer, H.A.; Ahmed, W.M. and Shalaby, S.I. (2000): Effect of chronic low dose oral administration of the OPC Selecrone on some blood constituents and reproductive parameters in Baladi sheep. Egypt.J. Comp. Pathol. , Clin. Pathol. , 13: 81-88.

Bal, H.S. and Mungkornkarn, P. (1978): Chronic toxicity effects of methoxychlor on the reproductive system of the rat. Proc. Int. Cong. Toxicol. ,1: 446-447.

Bonney, R.C.; Furr, J. A. and Cunningham, F.J. (1973): Radioimmunoassay of lutenizing hormone and progesterone during the ovulatory cycle of the hen (Gallus domesticus). Vet. Rec., 93:76-77.

Boukhlig, R.; Adams, N.R. and Martin, G.B. (1996): Effect of nutrition on the balance of production of ovarian and pituitary hormones in ewes. Anim. Reprod. Sci. ,45: 59-70.

Cornu, C. and Cognie, Y. (1985): Utilization of Romnove sheep in a system of integrated husbandry. In Genetics of Reproduction in Sheep, edited by R.B. Land and D.W. Robinson, PP: 383-390. London: Butterworth.

Cumming, A.M. and Perreault, S.D. (1990): Methoxychlor accelerates embryo transport through the rat reproductive tract. Toxicol. Appl. Pharmacol., 102: 110-116.

Dinar, A.M.; Diskin, M.G.; McDonagh, T. and Sreenan, J.M. (1987): Oestrus and ovarian response in repeatedly superovulated cows. Theriogenology, 27: 201 (Abst)

Driancourt, M.A. and Fry, R.C. (1992): Effect of superovulation with P-FSH or PMSG on growth and maturation of the preovulatory follicles in sheep. Anim. Reprod. Sci., 27: 279-292.

Faundez, R.; Sitarska, E.; Klucinski, W. and Duszewska, A.M. (1996): The effect of persistent chlorinated hydrocarbons on the secretion of the estradiol and progesterone by bovine granulosa cells in vitro. Zentrabl. Vetcrinarmed. ,43: 317-323.

Geldard, H.; Scaramuzzi, R.J. and Wilkins, J.F. (1984): Immunization against polyandroalbumin leads to increases in lambing and tailing percentages. N. Z.Vet.J,. 32: 2-5.

Gustavsson, I. (1980): Chromosome aberrations and their influence on the reproductive performance of domestic animals. A Review Z. Tcirzuchtg Zuchtgoboil. ,97: 176.

Halnan, C.R. (1977): Autosomal deletion and infertility in cattle. Vet. Rec., 90:572.

Hassanene, M.S.; Abdoon, A.S.S. and Mahrous, K.F. (1995): Effect of hormonal treatment and cytogenatical aspects on ovarian function of Egyptian Baladi cattle heifers suffering from gonadal dysgenesis with special reference to serum progesterone levels. Vet. Med. J. Giza, 43: 257-267.

Kafi, M. and McGowan, M. (1997): Factors associated with variation in the superovulatory response of cattle. Anim. Reprod. Sci., 48: 137-152.

Krause, W. and Homola, S.C. (1974): Alteration of seminiferous epithelium and Leydig cells of the rat testis after application of dichlorovos. Bull. Environ Toxicol. Contam., 11: 429-433.

Landolt, M.L. and Kocon, R.M. (1983): Fish shell cytogenetic-a measure of genotoxic effects of environmental pollutionís. In Aquatic Toxic (J.O. Nrigu, ed.), John Wiley and Sons Inc., PP: 335-352.

Laws, S.C.; Carey, S.A.; Hart, D.W. and Cooper, R.L. (1994): Lindan does not alter the estrogenic receptor or the estrogen-dependent induction of progesterone receptors in sexually immature or ovarectomized adult rats. Toxicology ,92: 127-142.

Lopaz-Barbella, S.R.; Warnick, A.C.; Wise, T.H. and Field, M.J. (1979): Endocrine regression by prostaglandin F2(α. J. Anim. Sci., 48:1135-1142.

Mahrous, K.F. and Abdoon, A.S.S. (1996): Relationship between superovulation and cytogenetical abnormalities in buffalo. Egypt.J. Appl. Sci., 5:139-147.

Martinez, .M. and Swartz, W.J. (1991): Effects of methoxychlor on the reproductive system of the adult female mouse. 1: Gross and histological observations. Reprod. Toxicol., 5:139-147.

McNatty, K.P.; Hudson, N.; Gibb, M.; Ball, K.; Henderson, K.M.; Heath, D.A.; Lun, S. and Kieboom, L.E. (1985): FSH influence follicle viability, oestradiol biosynthesis and ovulation rate in Romney ewes. J. Reprod. Fert., 75:121-131.

Naqvi, S.M. and Gulyani, R. (1998): The effect of gonadotrophin releasing hormone and follicle stimulating hormone in conjunction with Pregnant mare serum gonadotrophin on the superovulatory response in crossbred sheep in India. Trop. Anim. Health Prod., 30:369-376.

Pratt, S.L.; Spitzer, J.C.; Burns, G.L. and Phyler, B.B. (1991): Luteal function, estrous response and pregnancy rate after treatment with norgestomet and various dosages of estradiol valerate in suckled cows. J. Anim. Sci., 69: 2721-2726.

Rubianes, E.; Ungerfeld, R.; Vinoles, C.; Rivero, A. and Adames, G.P. (1997): Ovarian response to gonadotropin treatment initiated relative to waves emergence in ultrasonographically monitored ewes. Theriogenology 47: 1479-1488.

Schmutz, S.M.; Moker, J.S.; Barth, A.D. and Mapletoft, R.J. (1991): Embryonic loss in superovulated cattle caused by the 1; 29 Robertsonian translocation. Theriogenology 35: 705-714.

Snedecor, G.W. and Cochran, W.G. (1980): Statistical Methods. 7th ed. Iowa State University Press. Ames Iowa, USA.

Tiemann, U. and Kuchenmeister, U. (1999): Influence of organochlorine pesticides on ATPase activities of microsomal fractions of bovine oviductal and endometrial

cells. Toxicol. Lett., 104: 75-81.

Tiemann, U. and Pohland, R. (1999): Inhibitory effects of organochlorine pesticides on intrcellular transfer of Lucifer Yellow in cultured bovine oviductal cells. Reprod. Toxicol., 13: 123-130.

Tiemann, U.; Pohland, R. and Schneider, F. (1996): Influence of organochlorine pesticides on physiological potency of cultured granulosa cells from bovine perovulatory follicles. Theriogenology 46: 253-265.

Tokos, M. and Tokosova, M. (1990): The effect of season on the ovarian response in sheep after administration of the serum gonadotropin PMSG. Vet. Med. (Praha), 35:231-236.

Villagomez, D.A.; Andersson, M.; Gustavsson, I. and Ploen, L. (1993): Synaptonemal complex analysis of a reciprocal translocation, rcp (20; 24) (q17; q25), in a subfertile bull. Cytogenet Cell Genet. ,62: 124-130.

Weishun, H.E.; Aihua, L.I.U.; Haixian, B.A.O. and Chen, Y. (1982): Effects of 7 pesticides on chromosome aberration, SCE and cell cycle kinetics changes in cultured red muntjac cells. Zool. Res. ,3: 129-136.