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Occurrence of Organochlorine Compounds in Follicular Fluid and Their Effect on Steroid Hormones in Follicles of Camels

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ABSTRACT

Farm animals may be exposed to environmental pollutant. Occurrence of organochlorines compounds in follicular fluid and their effects on steroid hormones were studied in camels. Insecticide was determined in follicular fluid collected from 100 camels. About 11 and 12% of camels surveyed have 0.83±0.03 and 0.25±0.02 ng mL^{-1} endosulfan and hepatochlor epoxide, respectively in their follicular fluid. Other organochlorine compounds concentration was below detection limit of the assay. Follicles with detectable hepatochlor and endosulfan have significantly lower concentration of estradiol and testosterone. It is suggested that organochlorine may inhibit the synthesis of follicular steroids.

Key words: Organochlorine, insecticides, pollution, follicular fluid, steroids, camels, Saudi Arabia

INTRODUCTION

Environmental pollutants such as organochlorine compounds are widely used in agriculture and industry. They present in the air, ground water and soil and are found in and on plants. Because of their physiochemical properties, they accumulate in animal and human tissues (Lossifidou *et al*., 1999). Their occurrence is related to human activities (Baek *et al*., 1991; Al-Mutlaq *et al*., 2002) and may cause health effects (Baek *et al*., 1991; Menichini, 1992). They are categorized by the agency of toxic substance and disease registry (ATSDR, 1994) as probably carcinogenic to human and animals.

Farm animals such as camels may be exposed to varying concentrations of environmental pollutants for extended period of times. Possible effects of these exposures on the fertility of livestock would be of commercial importance (Beard and Rawlings, 1998). Furthermore, in case animals undergoing assisted reproduction technologies, organochlorine levels measured in follicular fluid are likely to be a biologically relevant marker of exposures since it reflects the ovarian microenvironment and thus organochlorine exposure of the maturing oocyte. Environmental pollutants have been found in human follicular fluid among other human tissues and body fluids, the presence of organochlorine and dioxins (Tsutsumi *et al*., 1998) was shown by some investigators. There is little information available on the presence of pollutants in the follicular fluid of farm animals (Faundez *et al*., 1996; Kamarianos *et al*., 2003). Follicular and endocrine environment affect oocyte quality, steroid hormones and their receptors. Any interference with follicular function is likely to impair reproductive health. This study was conducted to investigate the presence of organochlorine compounds and steroid hormones in follicular fluid of camels raised in Eastern Region of Saudi Arabia.

MATERIALS AND METHODS

Collection of follicular fluid: Ovaries were collected from 100 camels (5-8 years old) raised on local farms during May-August 2011 and killed at two slaughter-houses in Al-Ahsa and Dammam within 1 h of the slaughter of the animal and placed in ice. The follicles were aspirated aseptically with a 19 gauge needle. The samples were centrifuged at 1500 g for 20 min at 4°C. The supernatant was stored at -20°C until assay.

Analytical methods: Samples were extracted and assayed according to the methods of Frank *et al.* (1985) and Homeida (2008). The compounds detected were hexachlorocyclohexane (HCH), endosulfan, lindane, hepatochlor, methoxychlor, dieldrin, aldrin and total DDT.

Insecticides residues were measured by electron capture (63 Ni source) gas chromatography (Hitachi, Japan) using 15 m×0.25 mm capillary column coated with a 0.24 μm thickness of SE-10. Chromatographic conditions are as follows: injector temperature, 225°C, detector temperature, 300°C; column oven, programmed for an initial 1 min^{-1} hold at 90°C followed by 15°C min^{-1} to 20°C and the 5°C min^{-1} to 25°C, carrier gas helium at 20 cm sec^{-1} (105 kPa head pressure) and 30 mL argon methane (95+5)/min make-up gas to the detector. The injection was auto injection of 1.5 μL 1 with capillary inlet system configured in the splitless mode with the by-pass valve held open for 0.5 min.

Recoveries were determined by direct fortification of serum with known concentration of compounds in acetone and analyzed as described above. Results were corrected for extraction losses. Detection limits of the assay else and the recovery values are shown in Table 1.

Steroid hormones were measured by radioimmunoassay (Homeida *et al.*, 1988). The intra-assay co-efficient of variation (CV) for estradiol was 9.3% and for testosterone was 11%. The interassay CV for estradiol was 7.1% and for testosterone was 5.4%.

Data analysis: For the comparison of the concentrations of organochlorines and hormones fluid kruskal-wallis and Mann-Whitney nonparametric testes were used. Statistical analysis was performed using SPSS 9.0 and a probability of $p = 0.05$ was the minimum level of significance.

RESULTS AND DISCUSSION

About 12% of camels have 0.25±0.02 ng mL^{-1} hepatochlor epoxide in their ovarian follicles. Only 11% of camels have 0.83±0.02 ng mL^{-1} endosulfan in their follicles. Other organochlorine compounds were below the detection limit of the assay (Table 2).

Follicles with detectable hepatochlor and endosulfan have significantly ($p<0.001$) lower concentrations of estradiol (18.3-20.1 pg mL^{-1}) and testosterone (51.2-54.1 pg mL^{-1}) compared to follicles with no detectable organochlorine compounds (Table 3). About 23% of camels surveyed have detectable organochlorine compounds in their ovarian follicles, mainly hepatochlor epoxide and endosulfan. The follicular membrane is readily permeable to low and high molecular substance that can diffuse into follicular fluid (Edwards, 1974). Insecticides were detected in follicular fluid of cattle, sheep, goats and pigs (Kamarianos *et al.*, 2003) and women (Meeker *et al.*, 2009); hepatochlor and endosulfan being among the most organochlorine detected in follicular fluid. However, lower concentrations were seen in our study than those reported for human (Meeker *et al.*, 2009) and animals (Lossifidou *et al.*, 1999). Furthermore, the present levels were also below the maximum residue limit for fat tissues (200 ng g^{-1}) for both insecticides according to European regulations (EEC, 1986).

Table 1: Detection limits and recovery values for insecticides assay

Analyte	Detection limit (ng mL^{-1})	Recovery (%)
Total DDT	0.4	88
HCH	0.1	89
Methoxychlor	1.0	83
Heptachlor epixode	0.1	90
Dieldrin	0.1	82
Aldrin	0.2	81
Endosulfan	0.1	87
Lindane	0.1	92

Table 2. Mean±SD concentration (ng mL^{-1}) of organochlorine compounds in follicular fluid in camels

Analyte	Concentration of organchlorine (ng mL^{-1})	Frequency (%)
Total DDT	n.d	
HCH	n.d	
Methoxychlor	n.d	
Hepatochlor epoxide	0.25±0.02	12
Dieldrin	n.d	
Aldrin	n.d	
Endosulfan	0.83±0.03	11
Lindane	n.d	

n.d = below detection limit

Table 3: Mean values (± SD) for concentration of steroid hormones in follicular fluid of camels exposed to organochlorine insecticides

Type of follicle (n)	Estradiol 17α (pg mL^{-1})	Testo sterone (pg mL^{-1})
Follicles with no detectable organochlorine (77)	32.4±1.3a	90.3±2.1a
Follicles with detectable heptachlor (12)	18.3±0.9b	54.1±1.6b
Follicles with detectable endosulfan (11)	20.1±0.8b	51.2±1.1b

Values in the same column with different superscript differ significantly at $p<0.001$

The frequency of contamination may be related to the geographic area, being around a big industrial area in Eastern region of Saudi Arabia. Occurrence of pesticides and herbicibes in soil and sand dust from Riyadh city, Saudi Arabia has been confirmed (Al-Mutlaq *et al*., 2002; Rushdi *et al*., 2004). Local agricultural application of these pesticides may be the main source of compounds in the dust of city of Riyadh and elsewhere in the kingdom. Uses of these pesticides and insecticides on camles, however, could not be ruled out. Evidence of organochlorine pesticides contaminating poultry tissue in Saudi Arabia has been reported (Homeida, 2008).

Follicular fluid with detectable hepatochlor and endosulfan contained significantly lower values of estradiol and testosterone compared to follicles with no detectable organochlorine compounds. Thus observation suggests that organochlorines could possibly inhibit the production of follicular steroid hormones. Exposure of bovine granulose cells *in vitro* of organochlorine compounds resulted in a decrease of estradiol production (Faundez *et al*., 1996; Tiemann *et al*., 1996), probably due to a decrease in the rate of steriodogensis (Sircar and Lahiri, 1990). An inhibitory effect of organochlorine on the expression of enzymes responsible for steroid synthesis and on estradiol and testosterone production in ovarian follicles was also noted (Gregoraszczuk *et al*., 2011), suggesting that in the camels organochlorines may affect the aromatizing pathway that catalizes testosterone into estrogens (Homeida and Cooke, 1984). Further studies on direct effect of organochlorine on granulose cells are required to confirm this point.

CONCLUSION

From the present study it has been concluded that extensive usage of insecticide may result in their increased intrafollicular concentration that inhibit synthesis of steroids and possible effects of these exposure on fertility of camels.

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