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Effects of Reproductive Status, Ovariectomy and Sex Steroid Administration on Estrogen and Progesterone Receptors in the Uterus of Camel

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Abstract: Sections of camel uterus stained with immunoperoxidase for demonstration of estrogen and progesterone receptors showed that receptors for the two hormones were confined to epithelial nuclei. Staining intensity was stronger for progesterone receptors during estrus and pregnancy but weaker for estrogen receptors during pregnancy. In ovariectomized camels intramuscular administration of estradiol benzoate at a dose of 5 mg animal^{-1} increased uterine progesterone and estrogen receptor concentrations. Administration of progesterone at a dose of 100 mg per animal decreased steroid receptor concentrations. It is suggested that in the camel, uterine steroid receptors are up-regulated by estradiol and down-regulated by progesterone.

Key words: Sex steroids, receptors, uterus, camels, estrogen, progesterone

INTRODUCTION

Camels are induced ovulators requiring copulation to trigger the ovulatory process (Musa and Abusineina 1978; Homeida *et al*., 1988). In the absence of an ovulatory stimulus, ovarian activity has been proposed to occur in waves of follicular growth and regression (Al-Eknah *et al*., 1993). Attempts to induce ovulation using GnRH (Homeida *et al*., 1991) or pregnant mare serum gonadotrophin, Elias and Cohen (1985) have resulted in a short-lived corpus luteum and uterine factors are suggested to be reason by Zoller *et al*. (1993). The uterus is able to sequester large amounts of circulating ovarian steroids due to the presence of specific high-affinity receptor proteins. Estrogen and progesterone modulated the activities of the uterus during the estrous cycle and pregnancy. Circulating concentrations of these hormones appear to regulate receptor concentrations within the endometrium. Concentration binding sites are highest in estrus and early diestrus and decrease later in diestrus (Tomanelli *et al*., 1991). Recent immuno histochemical studies employing monoclonal antibodies to estrogen and progesterone receptors and enucleation experiments have indicated that both receptor types are localized principally in the nucleus (King and Greene, 1984). Information on cellular availability of hormone receptors can lead to a better understanding of tissue changes during normal estrous cycle and pregnancy. This study was carried out to determine the cellular distribution of estrogen and progesterone receptors in the camel uterus during estrous cycle and pregnancy and treatment with sex steroids in ovariectomized animals.

MATERIALS AND METHODS

Animals: Twenty mature (4-5 years) female camels were kept in outdoor pens at the camel research Center, King Faisal University. They were given hay and water *ad libitiun*. A male was housed in the vicinity for estrus detection; females were considered to be in estrus but mating was not allowed (group 1, 4 animals). In some animals mating was allowed and if serum progesterone values exceeding 1 ng mL^{-1}, animals were considered pregnant (group 2, 4 animals). Twelve animals were ovriectromized (Homeida *et al*., 1991) and treated with either 100 mg progesterone (IM, group 4, 4 animals), 5 mg estradiol benzoate (IM, group 4, 3 animals) or 5 mL saline (IM group 5, 4 animals).

Jugular vein blood was obtained by venepuncture. Serum was separated and stored at -30°C until analysis for hormones.

Animals were slaughtered and uterine tissue were collected, quick-frozen in liquid nitrogen and stored at -70°C until receptor analysis.

Determination of progesterone and estrogen receptors: Tissue sections were cut onto gelatin-coated slides using a cryostat microtome. The ERICA kit (Abbot Laboratories, Abbot Park, ILL and USA) for immunocytochemical

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localization of estrogen receptors in breast tumors was modified for use in uterine tissue (Zaino *et al*., 1989). Briefly, the tissue was fixed in 10% formaldehyde. The primary antibody was a rat monoclonal anti-human estrogen receptor 1 gG and was used at a dilution of 1:10. The avidin-biotin complex staining procedure was performed according to the manufacture's instructions and the sections were then dehydrated and mounted. Progesterone receptor 1 gG was used at a dilution of 1:50. Slides were incubated with primary antibody for 2 h. Avidin and biotinylated peroxidase kits (vectastain) were purchased from Vector laboratories, (Burlingame, CA, USA) and the staining procedure was performed according to the manufacturer's instructions.

Controls antibodies (mouse 1 gG) were applied on parallel sections. The rabbit was used as control in every assay. Since the rabbit uterus was relatively rich in steroid receptors and steroid receptor distribution within the endometrial was well documented (Zaino *et al*., 1989). Staining was described as negative (0), trace (1+) weak (2+) moderate and 3+ = strong, according to the definition of Zaino *et al*. (1989) for the rabbit uterus.

Determiantion of steriod concentration: Progesterone and estrogen concentration in serum were determined according to radioimmunoassay methods previously validated and published (Homeida *et al*., 1988). The intra and interassay co-efficient of variation for progesterone were 4.2% (n = 25) and 11.2% (n = 20) and for estradiol 17β were 7.2% (n = 15) and 9.6% (n = 12).

Statical analysis: A statistical computer software program (Statistics for Windows, Analytical Software, Tallahassee, Fl) was used to perform the statical tests. The non-parametric kruskall Wallis test was used to determine whether there is any significant difference in staining throughout the estrus cycle or pregnancy or between different cell groups. The spearman rank correlation test was used for determination of correlations between the immunohistochemical scores and the serum hormone levels.

RESULTS

Staining with immunoperoxidase for progesterone and estrogen receptors was limited to epithelial cell nuclei and no staining of cytoplasm was observed in Table 1. During estrus (group 1) staining intensity for progesterone receptors was stronger in stroma ($p<0.01$) than in other parts of the uterus but weaker in luminal and glandular epithelium and myometrium. Staining intensity for estrogen receptor during this period is also stronger ($p<0.01$) in luminal and weaker in stromal epithelium and myometrium. In pregnant animals (group 2) staining intensity was stronger ($p>0.01$) in luminal, moderate in glandulat and stroma but traces or absent in myometrium. Intensity for estrogen receptors was weak or absent during pregnancy. Ovariectomy (group 5) resulted in weak intensity for estrogen and progesterone receptor in endometrial and myometrium. Administration of progesterone (group 3) to ovariectomized animals did

Table 1: Effects of reproductive status, ovariectomy and sex steroids administration on uterine distribution of Progesterone (PR) and Estrogen Receptors (ER) in camels

	Endometrium							
	Luminal		Glandular		Stroma		Myometrium	
Treatment group	PR	ER	PR	ER	PR	ER	PR	ER
Group 1 (Estrus)								
1	1+	3+	1+	3+	3+	1+	1+	1+
2	1+	3+	1+	3+	3+	1+	1+	1+
3	1+	3+	1+	3+	3+	1+	1+	1+
Group 2 (Pregnancy)								
1	1+	1+	1+	Trace	3+	Trace	Trace	0
2	1+	1+	2+	Trace	3+	Trace	+1	0
3	1+	1+	1+	Trace	3+	Trace	+1	0
Group 3 (Ovariectomized and progesterone treated)								
1	1+	1+	1+	Trace	1+	Trace	0	0
2	1+	1+	1+	Trace	1+	Trace	0	0
3	1+	1+	1+	Trace	1+	Trace	Trace	0
Group 4 (Ovariectominzed and estradiol treated)								
1	2+	1+	2+	2+	3+	2+	1+	1+
2	2+	1+	2+	3+	3+	2+	1+	1+
3	2+	1+	2+	3+	3+	3+	1+	1+
Group 5 (Ovariectomized and saline treated)								
1	1+	1+	1+	1+	1+	0	0	0
2	1+	1+	1+	1+	Trace	1+	1+	1+
3	1+	1+	1+	1+	0	0	0	0

Staining intensity: 0 = negative, 1+<weak, 2+ = moderate, 3+ = strong

Table 2: Mean±SD peripheral serum concentration of estradiol 17β and progesterone in camels

Animals	Estradiol 17β (pg mL^{-1})	Progesterone (mg mL^{-1})
Group 1 (Estrus)	62±8	0.1±0.03
Group 2 (Pregnant)	11.3±2.1 *	3.4±1.3*
Group 3 (Ovariectomized and progesterone treated)	7.2±1.6	6.3±0.2**
Group 4 (Ovariectomized and estradiol benzoate treated)	72.3±7**	<0.1
Group 5 (Ovariectomized and saline treated)	6±1	<0.1

*Values in the same column are different from those in groups 1; **Values are different from those in group 5 at $p<0.001$

not improve this situation but that of estradiol benzoate (group 4) caused stronger ($p<0.01$) intensity for estrogen and progesterone receptors in all parts of the uterus, compared to results in group 5 animals.

Concentration of steroid hormones is given in Table 2. High levels of estradiol and low levels of progesterone was observed during estrus (group 1). In contrast, low levels of estrogen and high level of progesterone was observed during pregnancy (group 2) which correlates ($r = 0.91$, $p<0.001$) well with staining for steroid receptors.

Administration of progesterone to ovariectomized animals (group 3) resulted in a significant ($p<0.001$) increase in serum concentration of progesterone and administration of estradiol benzoate (group 4) resulted in a significant ($p<0.001$) increase in serum concentration of estrogen compared to ovariectomized saline treated animals (group 5). High correlation ($r = 0.95$, $p<0.001$) was seen between increased levels of estrogen and staining intensity for progesterone and estrogen receptors in different parts of the uterus.

DISCUSSION

The present study shows that progesterone and estrogen receptors in cameline uterus are confined to epithelium nuclei. Similar results have been reported for rat (Gee *et al*., 1990), monkey (Brenner *et al*., 1991), human (lessey *et al*., 1988), ewe (Cherry *et al*. 1991), rabbit (Zaino *et al*., 1989) and mare (Watson *et al*., 1992). During estrus, staining intensity for progesterone receptors was strong in epithelial stroma but moderate in luminal and glandular epithelium. Staining intensity of estrogen receptors during this period was strong in luminal and weak in stromal epithelium and myometrium. High concentration of serum estrogen was observed during estrus, probably required for induction of progesterone receptor synthesis and eventually progesterone dominance over the uterus (Zoller *et al*., 1993). Similar steroidal distribution was observed in rabbit uterus (Zaino *et al*., 1989). It is interesting that both camels and rabbit are induced ovulator (Homeida *et al*., 1988) having continueous follicular growth during estrous cycle (Al-Eknah *et al*., 1993) and expected to produce similar factors that affect uterine steroid receptor distribution (Zaino *et al*., 1989). However, pattern of receptor distribution during estrus is different from that of mare (Watson *et al*., 1992), primates (Brenner *et al*., 1991) and rat (Gee *et al*., 1990), which illustrates existence of species differences.

During pregnancy the pattern of progesterone receptor distribution was similar to estrus period but pattern of estrogen receptors was suppressed may be as a feature of pregnancy recognition in this species as sampling was performed during early pregnancy. In these animals higher levels of progesterone and lower levels of estrogen were observed.

Administration of estradiol benzoate to ovariectomized camels increased staining intensity and that of progesterone decreased staining intensity for uterine steroid receptors that well correlated with levels of sex hormones, compared to ovariectomized saline treated animals. In many tissues, progesterone inhibits replenishment of estrogen receptors and this curtails estrogen action (West *et al*., 1986). Furthermore, it has been shown in many animals species that steroidal receptors are up-regulated by estradiol and down-equaled by progesterone (Ogle *et al*., 1989).

CONCLUSION

The present study shows that in the camel, sex steroid receptors are confined to uterine epitheli nuclei. Their concentration are well correlated with serum steroidal levels in this species.

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