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Gonadotropin-Releasing Hormone-Like Factors in the Seminal Plasma of the Arabian Camel (*Camelus dromedarius* L.)

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ABSTRACT. The presence of gonadotropin-releasing hormone (GnRH)-like factors in the seminal plasma of the Arabian camel (*Camelus dromedarius* L.) has been studied in immature rats. Immature male and female rats treated with the Arabian's seminal plasma (SP) or with synthetic GnRH developed heavier testes and ovaries and large seminiferous tubules than those of the controls. Immature female rats treated with GnRH and those injected with highest two doses of the camel's SP has developed a greater number of ovarian follicles than those of the corresponding controls. Those receiving the lower three doses of the camel's SP has developed a greater number of small ovarian follicles than in their controls. It is concluded that the Arabian camel's SP contains an ovulation stimulation factor.

Gonadotropin-releasing hormone (GnRH) is a hypothalamic hormone controlling the release of luteinizing hormone (LH), and of the follicle stimulating hormone (FSH) from the pituitary gland (Matsuo *et al.* 1971 and Bragus *et al.* 1972). This hormone as well as GnRH-like factors have been also detected in the pituitary gland (Beyeot *et al.* 1984 and Li *et al.* 1984), in the pineal gland (Reiter 1980) of several vertebrates, as well as in the seminal plasma of the bull and humans (Majunath 1984 and Sokol *et al.* 1985). The physiological roles of these factors may be similar to that of GnRH since they could: cross-react with specific antisera raised against GnRH (Sharpe and Fraser 1980), replace iodine-labelled GnRH in radioimmunoassays and radioreceptor assys specific for GnRH (Sharpe *et al.* 1981), and stimulate the release of LH and FSH from pituitary tissue cultured *in vitro* (Ying *et al.* 1981).

Since the Arabian camel is an induced ovulator that exhibits follicular cycles with follicles developing and regressing successively, with ovulation induced only by using one of the following methods: 1) by mating (Shalash and Nawito 1964, Novoa 1970, Musa and Abu sineina 1978, Homeida *et al.* 1988); 2) by depositing the whole semen or sperm free seminal plasma into the uterus (Chen *et al.* 1990, Musa *et al.* 1992); 3) by intramuscular (im) injection of human Chorionic Gonadotropin (hCG) or GnRH (Chen *et al.* 1985). Furthermore, Marie and Anouassi (1987) observed that plasma LH levels rise sharply to a peak about 3 to 4 h after copulation in Arabian camel. Similarly, Xu *et al.* (1985) demonstrated the occurrence of a preovulatory LH peak in the blood of bactrian camels at 4 h post insemination, with ovulation occurring at 36 to 48 h. Chen *et al.* (1985) and Zhao *et al.* (1990) demonstrated that an ovulation-inducing factor is present in the seminal plasma of bactrian camels. Hence, the present study has been formulated to investigate the presence of such factors in the seminal plasma of the Arabian camel.

Materials and methods

Semen collection and treatments

A male Arabian camel (Majaheem) of proven high fertility was chosen for semen collection and was kept at the livestock farm of our Department in Riyadh, where it has been maintained on concentrates and roughages (the concentrate mixture was containing 13.4% digestible protein and 72% total digestible nutrients, while the fodder was alfalfa). Water was available at all times in a drinking basin and salt licks were provided *ad libitum.* Semen samples were collected from that camel using an artifical vagina obtained from IMV, Cassou, France. The seminal plasma was then separated from the spermatozoa by centrifugation at 1500 g for 30 min under cooling at 4 °C and was stored at –20 °C until used later.

Experimental rats

Forty male and 40 female immature Wistar albino rats, 21 days old and with a mean weight ± standard deviation of 47.4 ± 0.22 g for males and 37.7 ± 0.26 g for females were used. The rats were divided equally into 8 groups, each group containing 5 males and 5 females. Group 1 was injected im with saline (vehicle) to serve as controls, while groups 2 to 7 were injected im twice daily for 4 days with 25, 50, 75, 100, 125, 150 µl seminal plasma, respectively. Group 8 received 5 µg of synthetic GnRH (Fertagyl, Intervet, Boxmeer, Holland) at the same intervals. On Day 5, all rats were killed and the testes and ovaries were removed, weighed and sectioned by standard histological techniques (Humason 1967). Ovarian follicles were counted and classified according to their diameter (small < 100 µm, medium 100-200 µm, and large follicles > 200 µm) and the diameter of the seminiferous

tubules was assessed for circular or nearly circular tubules in profile.

The data were statistically analysed using the GLM procedure of SAS (Goodnight *et al.* 1985).

Results

The live body weights of male and female rats increased with treatment by SP or GnRH; although not significant, the highest response was respectively obtained with 125 and 150 µl compared to the controls (68.4 ± 5.04, 72.2 ± 5.04 and 67.8 ± 5.04 vs. 61 ± 5.04 gm in males and 59.0 ± 4.56, 58.8 ± 4.56 and 57.8 ± 4.56 vs. 47.4 ± 4.56 in females, respectively). The weights of testes and ovaries has significantly increased ($p < 0.05$) in immature rats treated with SP or GnRH with highest responses being obtained with 125 and 150 µl of SP and with GnRH (5 µg) compared to the controls (Table 1).

Table 1. Variations in mean ± SD weight of testes and ovaries of immature rats treated with GnRH or with seminal plasma of Arabian camel (SP). Five rats included in each sex X treatment group (n = 5)

Treatment	Weight of	
	Testes (g)	Ovaries (g)
Control	0.76 ± 1.13^{b}	0.039 ± 0.004^{b}
GnRH (5 µg)	1.71 ± 1.13^{a}	0.053 ± 0.005^{a}
SP (µl):		
25	0.83 ± 1.13^{b}	0.054 ± 0.005^{a}
50	0.90 ± 1.13^{b}	0.052 ± 0.005^{a}
75	0.81 ± 1.13^{b}	0.043 ± 0.005^{ab}
100	1.09 ± 1.13^{a}	0.043 ± 0.005^{ab}
125	1.00 ± 1.13^{a}	0.057 ± 0.005^{a}
150	1.12 ± 1.13^{a}	0.057 ± 0.005^{a}

[a,b]Values within the same column bearing different superscripts differ significantly at $p < 0.05$.

The diameter of the seminiferous tubules has also significantly increased ($p < 0.05$) in immature rats treated with SP and GnRH, with highest responses being obtained with 50, 100 of SP and with GnRH (5 µg) compared to the controls (Table 2 and Fig. 1).

Table 2. Variations in mean ± SD number of ovarian follicles and seminiferous tubule diameter (STD) in immature rats treated with GnRH or with seminal plasma of Arabian camel (SP) (n = 5)

Treatment	Follicles Diameter			STD (µm)
	> 200 µm	100- 200 µm	< 100 µm	
Control	2.0 ± 0.72^{b}	2.0 ± 1.55^{b}	02.7 ± 2.52^{c}	36.00 ± 5.76^{b}
GnRH (5 µg)	5.3 ± 0.76^{a}	7.0 ± 1.55^{a}	11.0 ± 2.52^{b}	55.76 ± 5.76^{a}
SP (µl):				
25	3.0 ± 0.76^{b}	4.3 ± 1.55^{b}	22.0 ± 2.55^{a}	50.00 ± 5.76^{ab}
50	2.0 ± 0.76^{b}	4.0 ± 1.55^{b}	12.3 ± 2.52^{b}	61.67 ± 5.76^{a}
75	2.0 ± 0.76^{b}	8.0 ± 1.55^{b}	12.0 ± 2.52^{b}	48.33 ± 5.76^{ab}
100	6.7 ± 0.76^{a}	6.3 ± 1.55^{a}	07.3 ± 2.52^{b}	63.33 ± 5.76^{a}
125	4.0 ± 0.76^{ab}	6.3 ± 1.55^{a}	10.0 ± 2.52^{b}	44.00 ± 5.76^{ab}
150	7.3 ± 0.76^{a}	8.3 ± 1.55^{a}	11.7 ± 2.52^{b}	45.30 ± 5.76^{ab}

[a,b]Values within the same column bearing different superscripts differ significantly at $p < 0.05$.

The number of large follicles was significantly ($p < 0.05$) higher in immature rats treated with GnRH, 125 or 150 µl of SP while, that of small follicles was also higher in those treated with SP (25, 50 and 75 µl) compared to controls (Table 2 and Fig. 2).

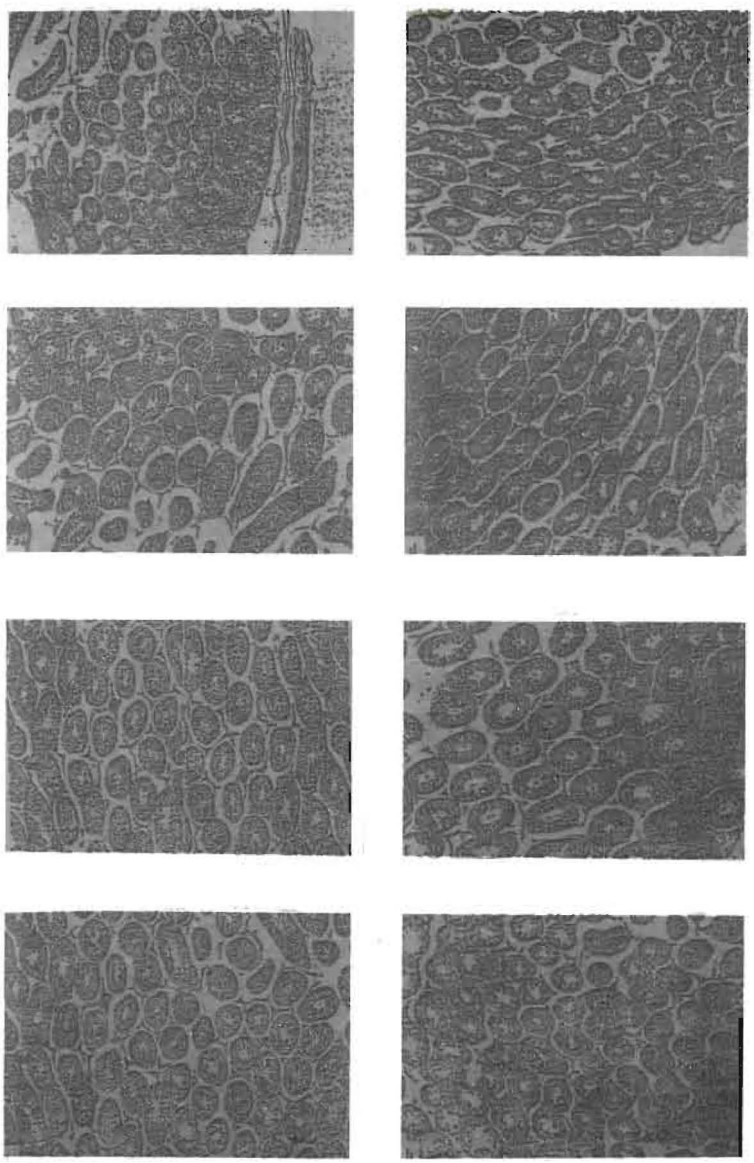

Fig. 1. Cross sections of rat testes; a, controls; b, treated with 5 µg of synthetic GnRH; c, d, e, f, g and h treated with 25, 50, 75, 100, 125 or 150 µl of seminal plasma, respectively.

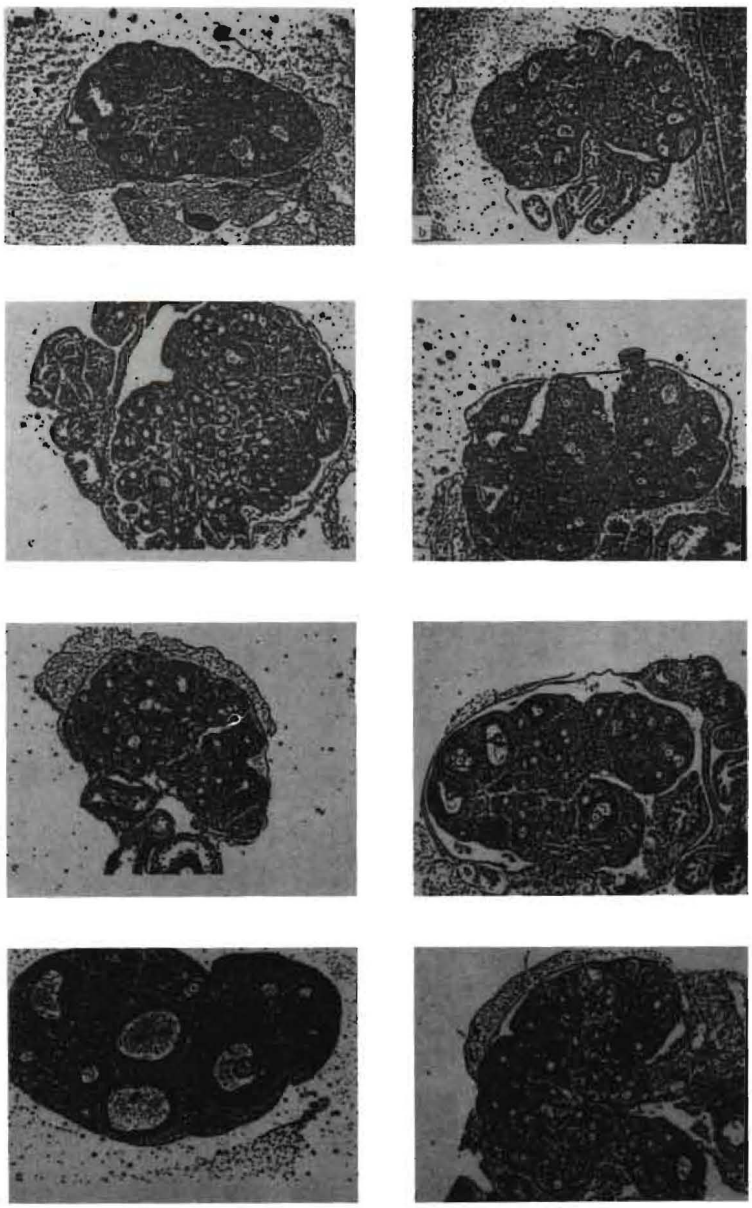

Fig. 2. Cross sections of rat ovaries; a, controls; b, treated with 5 μg of synthetic GnRH; c, d, e, f, g and h treated with 25, 50, 75, 100, 125 or 150 μl of seminal plasma, respectively.

Discussion

The results indicate that the effect of seminal plasma (SP) on live body weight and on the weight of testes and ovaries of immature rats was similar to that of GnRH treatment. Therefore, it is evident that the SP of the Arabian camel contains a biologically active component(s) similar in activity to GnRH. Moreover, SP and GnRH treatments have resulted in an increase of the diameter of the seminiferous tubules and have also increased the number of ovarian follicles. Such effect could also be attributed to GnRH-like component(s) in the camel's SP. The poor development of the seminiferous tubules in the control animals was probably caused by the combined effects of low plasma concentrations of FSH and low intratesticular concentrations of testosterone, since both these factors are critical for tubular growth and development of the testis (Kerr *et al.* 1992). The low number of developing follicles in the control may be due to the low level of FSH which is required for follicular development (Fortune 1994). Zhao *et al.* (1990) have observed that ovulation can be induced in the Bacterian camel by insemination or by the im injection of Bacterian camel SP, which they have attributed to the presence of an ovulation inducing factor in the SP of that camel. Such a factor was also observed to stimulate the release of LH and FSH in the peripheral blood (Xu *et al.* 1985 and Zhao *et al.* 1990), as well as *in vitro* Zhao *et al.* (1992). The present study is only a preliminary one and further studies are needed on large numbers of animals under semi-arid environments to investigate the effects of those factors.

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العوامل المشابهة للهرمون المحرر لمنبهات المناسل في البلازما المنوية في الإبل العربية

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لقد تم إجراء هذه الدراسة لاختبار وجود عوامل مشابهة للهرمون المحرر لمنبهات المناسل في البلازما المنوية في الإبل العربية ، حيث تم جمع عينات من السائل المنوي باستخدام مهبل صناعي مخصص للإبل وتم فصل البلازما المنوية وتخزينها في درجة حرارة ٢٠°م تحت الصفر لحين استخدامها . وفي الدراسة تم استخدام أربعون ذكر وأربعون أنثى جرذ حيث تم تقسيمها بالتساوي إلى ثماني مجموعات . استخدمت المجموعة الأولى مجموعة ضبط للمقارنة بينما حقنت المجاميع ٢ ، ٣ ، ٤ ، ٥ ، ٦ ، ٧ مرتين يوميا ولمدة أربعة أيام بالبلازما المنوية كما يلي : ٢٥ ، ٥٠ ، ٧٥ ، ١٠٠ ، ١٢٥ ، ١٥٠ ميكروليتر على التوالي . وحقنت المجموعة الثامنة يوميا ولمدة أربعة أيام بالهرمون المحرر لمنبهات المناسل المصنع (٥ ميكروجرام) . وفي اليوم الخامس تم قتل جميع الجرذان وإزالة الخصي والمبايض منها حيث تم وزنها وتشريحها . ثم بعد ذلك تم عد وتصنيف الجريبات المبيضية على أساس قطرها (صغيرة أقل من ١٠٠ ومتوسطة من ١٠٠-٢٠٠ وكبيرة أكبر من ٢٠٠ ميكرميتر) . هذا ولقد

أوضحت النتائج أن هناك زيادة في أوزان الخصي والمبايض وأيضا زيادة في أقطار الأنابيب المنوية في الجرذان التي حقنت بالبلازما المنوية أو بالهرمون المحرر لمنبهات المناسل المصنع مقارنة بمجموعة الضبط أيضاً كان عدد الجريبات الكبيرة اكثر في الجرذان التي حقنت بالهرمون المحرر لمنبهات المناسل المصنع أو بـ ١٢٥ أو بـ ١٥٠ ميكروليتر من بلازما الأبل المنوية بينما كان عدد الجريبات الصغيرة اكثر في المجاميع التي حقنت ٢٥ و ٥٠ و ٧٥ ميكروليتر مقارنة بمجموعة الضبط . دلت هذه النتائج على أن تأثير بلازما الإبل المنوية على الخصي والمبايض مشابه لتأثير الهرمون المحرر لمنبهات المناسل مما يدل على وجود عوامل مشابه للهرمون المحرر لمنبهات المناسل في بلازما الإبل العربية المنوية .