EFFECT OF LEPTIN ON OVOGENESIS DURING DEVELOPMENT IN FEMALE WISTAR RAT

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Abstract. The present study aims to investigate the effect of leptin treatment on ovarian weight and ovarian follicle enumeration during development in female Wistar rats. The experiment is carried out on 30 and 60-day-old females. Leptin diluted in 9‰ NaCl is injected daily for 5 days intraperitoneally at a dose of 8 µg / 100 g of body weight to the experimental females. The same volume of the NaCl solution is injected into the controls. Animals are sacrificed 24 hours after the last injection. Ovaries are removed and weighed. The right ovaries are fixed in 10% formaldehyde for histological study. The results reveal a non-significant increase in absolute and relative mean weights of the right and left ovaries in leptin-treated groups compared to controls in both immature and adult rats. The histological study reveals that in 30-day-old females, the number of atretic follicles is most important compared to normal follicles after leptin-treatment, which suggests that leptin appears to stimulate cellular apoptosis at immature age. These results allow us to conclude that leptin seems to influence ovarian weight and follicular atresia.

Key words: Leptin, ovarian weight, enumeration, follicle, atresia

INTRODUCTION

Reproductive disorders are partly related to nutritional imbalances and body reserves, particularly fat ones. Leptin is a non-glycosylated protein of 16 kDa, essentially secreted by adipocytes proportionally to body fat (Zhang et al., 1994). This cytokine is involved in many physiological processes related to the ubiquitous distribution of its Ob-R receptors. It plays a key role in the regulation of food intake and energy balance through a feedback mechanism on the hypothalamic centers of satiety and hunger. Indeed, leptin acts on homeostasis by decreasing food intake and promoting energy expenditure and thermogenesis. The detection of mRNAs in the human placenta in 1997 (Masuzaki et al., 1997) reversed the status of leptin as an adipocyte hormone implicating this adipokine in the physiology and endocrinology of reproduction (Messinis and Milingos, 1999, Pasquali and Gambineri, 2006).

Leptin has a central action on reproductive function at a certain concentration that appears to correlate with other metabolic signals, including glucose availability to regulate GnRH secretion (Foster and Nagatani, 1999). Its direct action on the gonads has been confirmed by the detection of the leptin receptor in the ovary (Klarlsson et al., 1997, Spicer and Francisco, 1997, Ryan et al., 2003, Muñoz-Gutiérrez et al., 2005, da Silveira Cavalcante et al., 2009) and testis (Hoggard et al., 1997, Ishikawa et al., 2007).

The present work aims to investigate the effect of leptin treatment on ovarian weight and ovarian follicle enumeration during development in female Wistar rats.

MATERIAL AND METHODS

Animals: Two groups of female Wistar rats are constituted: the first is composed of 30-day old animals, while the second includes 60-day old ones. Each group is divided into control (C) and experimental treated females (T). The control and the corresponding experimental female have almost the same initial body weight (BW). The number of animals by group is specified in Table 1. The animals are kept at ambient temperature and hygrometry, and are exposed to external lighting. Experimental procedures were approved by the Institutional Animal Care Committee of the National Administration of the Algerian Higher Education and Scientific Research (Ethical approval number: 98-11, Law of August 22, 1998) and were conducted according to recommendations edited in the "Guide for the Care and Use of Laboratory Animals".

Table 1.

Number of animals by group

30 days-old		60 days-old	
Control (C)	Leptin-treated (T)	Control (C)	Leptin-treated (T)
n = 12	n = 12	n = 9	n = 8

Experimentation: Leptin (lyophilised powder, Sigma-Aldrich®) diluted in 9‰ NaCl is injected intraperitoneally to the experimental females at the dose of 8 µg / 100 g of body weight. The same volume of the NaCl solution is injected into the controls. Animals are injected daily for 5 days and then sacrificed 24 hours after the last injection by decapitation. The right and left ovaries are removed and weighed. The right ovaries are fixed in 10% formaldehyde for histological study. Photographs are taken using a Hirocam® camera. Histological images are directly recorded on a computer via TSView software and then processed.

Statistical analysis: The results are expressed in number, percentage and mean ± SEM. Statistical analysis is performed using the Student's test. The level of significance is: $p < 0.05$.

RESULTS AND DISCUSSION

Ovarian weight. Our results reveal a non-significant increase in absolute and relative mean weights of the right and left ovaries in leptin-treated females compared to controls in both 30-day and 60-day-old animals (Table 2 and Fig. 1, 2).

These results are similar to those of El-Shafaei et al. (2006) who report a significant increase in growth and weight of the right and left ovaries in leptin-treated females but contradict those of Panwar et al. (2012) who report that passive immunization against leptin, with or without gonadotropins, causes a significant increase in ovarian weight compared to control ovaries. In another study, immature 3-week-old mice were divided into four groups and received the following treatments by subcutaneous injections: (1) saline; (2) anti-leptin antibody (50 µg); (3) eCG or Equine Chorionic Gonadotropin (0.1 IU); (4) eCG (0.1 IU) + anti-leptin antibody (50 µg). No differences in ovarian weights were reported, but in the eCG + anti-leptin groups, the ovaries were heavier than those of control animals (McFarlane et al., 2014).

In *ob/ob* mice (obese, hyperglycemic, with thermoregulation disorders, hypogonadism and sterile), leptin injection causes a development of reproductive organs in both males (increase in weight of testes and seminal vesicle) and in females (increase in

weight of the ovaries and uterus) (Barash et al., 1996). In obese women, as in many obese mouse models, a hormonal profile of central hypogonadism has been reported. It would be related to the increased concentration of circulating estrogens (by the peripheral aromatization of androgens by excess adipose tissue) that inhibit Kisspeptin neurons, intermediates of steroid feedback on GnRH secretion. Leptin, ghrelin, and NPY may be involved in modulation of gonadotropic axis control via kisspeptins (Castellano et al., 2010).

Table 2.
Absolute and relative mean weights of the ovaries in control and leptin-treated females at 30 and 60 days of age (Values expressed in g ± SEM, NS: Nonsignificant, *p* = p-value)

			Right ovaries	*p*	Left ovaries	*p*	Right and Left ovaries	*p*
30 Days	Absolute weight	C	0.0336 ± 0.0032	*0.510* *NS*	0.0345 ± 0.0028	*0.736* *NS*	0.0680 ± 0.0057	*0.596* *NS*
		T	0.0373 ± 0.0045		0.0361 ± 0.0039		0.0733 ± 0.0081	
	Relative weight	C	0.0533 ± 0.0032	*0.296* *NS*	0.0554 ± 0.0028	*0.530* *NS*	0.1087 ± 0.0051	*0.322* *NS*
		T	0.0593 ± 0.0046		0.0578 ± 0.0027		0.1172 ± 0.0066	
60 Days	Absolute weight	C	0.1820 ± 0.0225	*0.762* *NS*	0.1903 ± 0.0225	*0.851* *NS*	0.3723 ± 0.0417	*0.925* *NS*
		T	0.1958 ± 0.0384		0.1838 ± 0.0255		0.3796 ± 0.0633	
	Relative weight	C	0.1243 ± 0.0136	*0.590* *NS*	0.1313 ± 0.0151	*0.929* *NS*	0.2556 ± 0.0262	*0.720* *NS*
		T	0.1409 ± 0.0267		0.1334 ± 0.0178		0.2743 ± 0.0439	

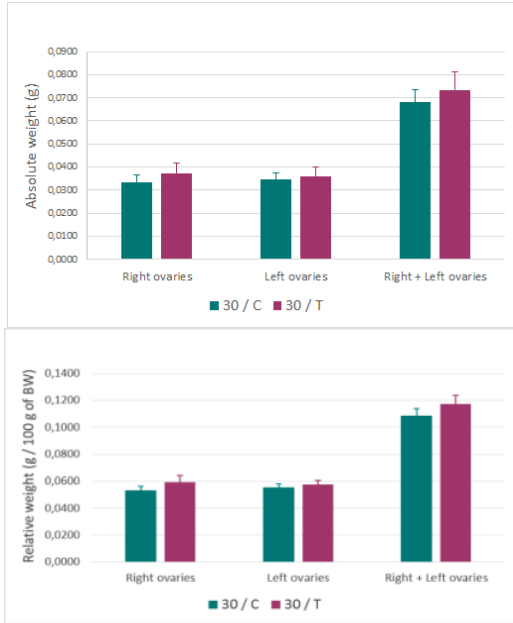

Figure 1. Absolute and relative weights of ovaries in control (C) and leptin-treated females (T) at the age of 30 days

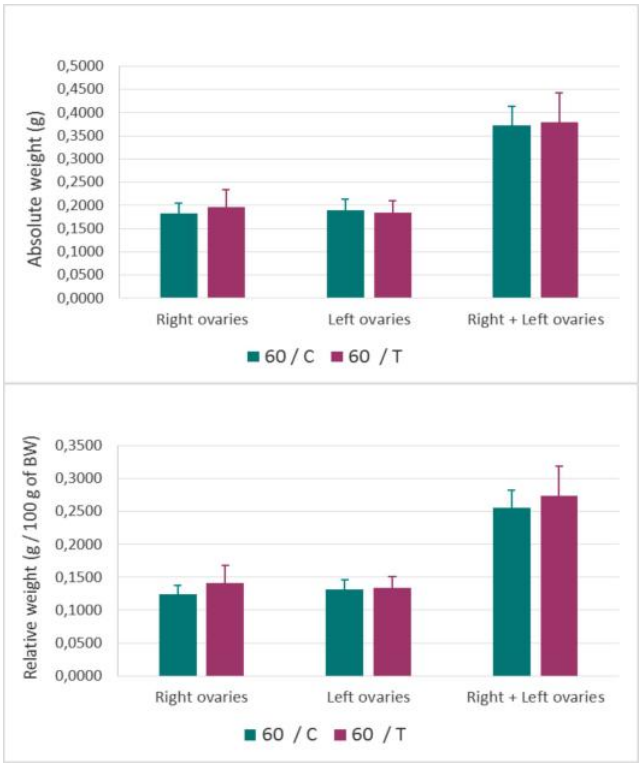

Figure 2. Absolute and relative weights of ovaries in control (C) and leptin-treated females (T) at the age of 60 days

Enumeration of follicles and corpus luteum

30 days Ovaries. The histological study reveals that in 30-days old females, the number of atretic follicles is more important after leptin treatment compared to normal follicles (276 vs 196 follicles, i.e. 47.18 vs 34.09%, $p > 0.05$), especially the tertiary and preovulatory follicles (Table 3, Fig. 3 & 4). Five corpus luteum were found on the ovary of a single experimental female.

Table 3.

Number and percentage of normal and atretic follicles by follicular stage in 30-day old control and experimental females

Follicles	30 / C				30 / T			
	Normal		Atretic		Normal		Atretic	
	Nb	%	Nb	%	Nb	%	Nb	%
Primordial	247	65.17	0	0	196	63.43	0	0
Primary	40	10.55	0	0	29	9.39	0	0
Secondary	37	9.76	11*	5.61	41	13.27	12	4.35
Tertiary	55	14.51	150**	76.53	43	13.92	202***	73.19
Preovulatory	0	0	35	17.86	0	0	62	22.46
Total	379	65.91	196	34.09	309	52.82	276	47.18

* *including 3 polyoocystes;* ** *including 8 polyoocystes;* *** *including 5 polyoocystes*

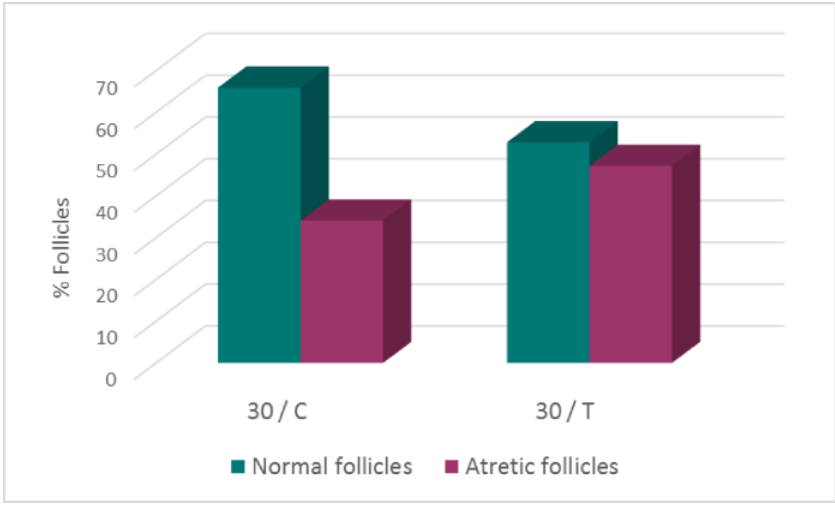

Figure 3. Percentage of normal and atretic follicles in 30 days ovaries

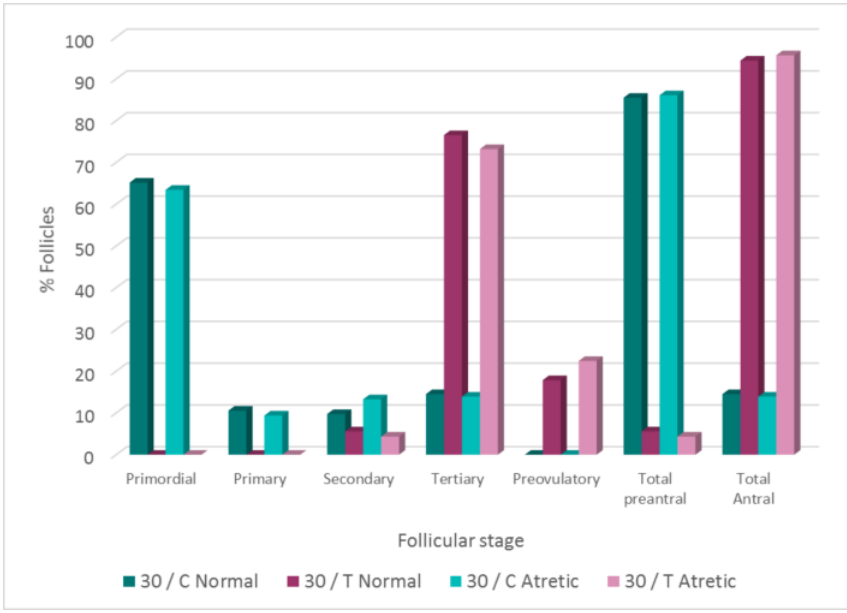

Figure 4. Percentage of normal and atretic follicles by follicular stage in 30 days ovaries

60 days Ovaries . In 60 days-old females, the number of atretic follicles appears to be less important after leptin-treatment compared to normal follicles (148 vs 158 follicles, i.e. 31.90% vs 43.05%, $p > 0.05$), particularly tertiary follicles (122 vs 140 follicles, $p > 0.05$) but not preovulatory ones (23 vs 17 follicles, $p > 0.05$, Table 4, Fig. 5 & 6). The mean number of corpus luteum between leptin-treated and control females is similar (59 vs 58 follicles).

Table 4.

Number and percentage of normal follicles by follicular stage in 60-day old control and experimental females

Follicles	60 / C				60 / T			
	Normal		Atretic		Normal		Atretic	
	Nb	%	Nb	%	Nb	%	Nb	%
Primordial	113	54.07	0	0	185	58.54	0	0
Primary	32	15.31	0	0	41	12.97	0	0
Secondary	41	19.62	1	0.63	61	19.30	3	2.03
Tertiary	23	11.00	140*	88.60	27	8.54	122	82.43
Preovulatory	0	0	17	10.76	2	0.63	23**	15.54
Total	209	56.95	158	43.05	316	68.10	148	31.90

* *including 1 polyoocyste ;* ** *including 2 polyoocystes*

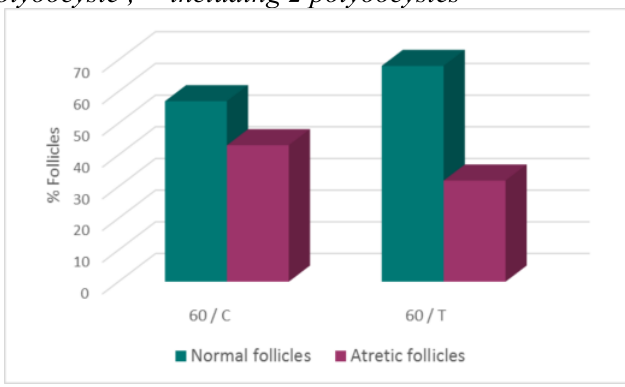

Figure 5. Percentage of normal and atretic follicles in 60 days ovaries

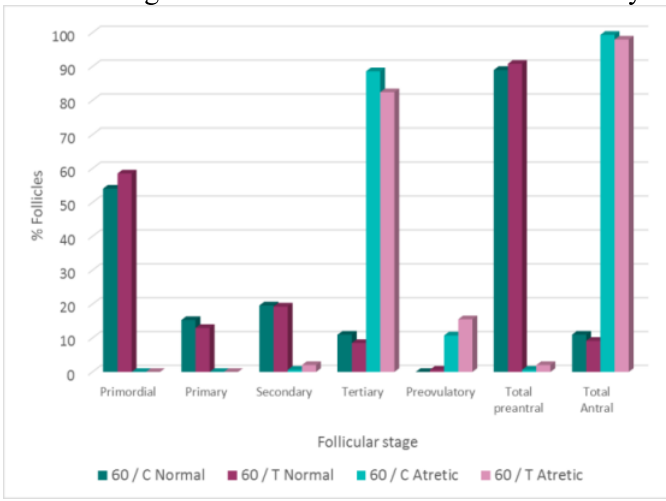

Figure 6. Percentage of normal and atretic follicles by follicular stage in 60 days ovaries

Atresia corresponds to the entrance into apoptosis of the follicle with hyalinization, fragmentation of the cytoplasm and thickening of the zona pellucida. The activation of Caspace 3 and the increase in expression of Bax are biomarkers of apoptosis. These two proteins are mainly expressed in atretic follicles, while Bcl2 (anti-apoptotic factor) is abundant in healthy preantral ones (Slot et al., 2006). Activation of caspase 3 is an

irreversible event. Our results related to follicular atresia show that leptin stimulates apoptosis at immature age. It seems to promote the elimination of incompetent oocytes (Mermillod et al., 2008). Indeed, the number of atretic preovulatory follicles after treatment is 62 vs 35 (p = 0.016 *). In addition, corpus luteum were found on the ovary of a single immature female which suggests that sexual maturity is not reached at this age in Wistar female rats.

CONCLUSION

Leptin injected intraperitoneally at a dose of 8 µg / 100 g of body weight daily for 5 days appears to influence ovarian weight in both immature and adult females. It also seems to have an effect on follicular atresia by preventing the occurrence of too early ovulation, before the maturation of the hypothalamic-pituitary-ovarian axis.

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