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Localization of resistin and its possible roles in the ovary of a vespertilionid bat, *Scotophilus heathi*

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ABSTRACT

The aim of the present study was to evaluate the expression and effect of resistin on ovarian activities of *Scotophilus heathi*. Immunohistochemical study showed marked variation in resistin immunostaining during different reproductive phases. Most intense immunostaining of resistin was noticed in thecal-interstitial cells in ovary during the period of delayed ovulation, the period of increased androgen synthesis and suppressed ovulation. The changes in ovarian resistin level also correlated positively with circulating leptin level and body white adipose tissue accumulation. The *in vitro* study showed that resistin alone preferentially stimulated progesterone synthesis, but with luteinizing hormone (LH) stimulated androgen secretion. Resistin alone dose-dependently increased expression of LH-receptor, steroidogenic acute regulatory protein and insulin receptor proteins in the ovary, whereas together with LH showed dose-dependent stimulatory effect on expression of androgen receptor and insulin receptor proteins in the ovary. In conclusion, during the period of fat accumulation increased ovarian resistin level may be responsible for increased androgen synthesis through insulin receptor mediated pathways in the ovary of *S. heathi*.

1. Introduction

Bats, the only flying mammals, belong to order chiroptera and they show several unique reproductive features such as, delayed ovulation, delayed implantation and delayed development. *Scotophilus heathi*, a seasonally monoestrous vespertilionid bat, shows the unique phenomena of delayed ovulation. *S. heathi* breeds once in a year from March to July. During the reproductive cycle of *S. heathi*, ovarian recrudescence (follicular development) occurs prior to winter dormancy in October, but the terminal maturation and ovulation of follicles are postponed until March [1]. The bat feeds vigorously around October leading to the heavy accumulation of fat prior to winter dormancy (late November to early February) [1,2]. Coinciding with the period of heavy fat accumulation during winter dormancy, the ovary contained many large antral follicles but all attempts to induce ovulation are unsuccessful [3]. The period of reproductive cycle when ovaries contain large follicles but ovulation does not occur is called the period of delayed ovulation [2]. During delayed ovulation, follicular development remained static, this is because of high production of androstenedione (A4) by the ovaries. [4]. High circulating level of androgen along with heavy accumulation of fat leads to suppressed follicular maturation and anovulation in *S. heathi*. Studies from our laboratory clearly suggest significant positive correlation between increased circulating androstenedione, insulin, body weight and fat deposition during the delayed phase in this bat species. Anovulation, hyperandrogenism and hyperinsulinemia with compensatory insulin resistance have been shown to be the characteristic feature of polycystic ovary syndrome (PCOS) [5–7] and more than 65% of women with PCOS are obese [8]. In the present study we have used bat model to explore the obesity associated reproductive disorders.

Obesity is a growing problem in all the regions of the world and has been shown to be associated with the several endocrine and metabolic anomalies. Adipose tissue secretes various adipokines like leptin, adiponectin and resistin, which have pleiotropic actions in the body. A direct role of leptin and adiponectin in ovarian function has been demonstrated in various mammalian species including bat species, particularly in *S. heathi* during the period of delayed ovulation [9–18]. Resistin, the adipokine, has been named after its property to induce insulin resistant condition in mice. Resistin is a cysteine rich protein and in humans it is encoded by the RETN gene [19]. Resistin level has been shown to be increased in

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Table 1
Details of the primary antibodies used for the immunohistochemistry/western blot study.

Antibody	Host species	Type and dilution	Source antigen	Source
Insulin receptor	Rabbit	Polyclonal, 1:1000	Human	Santacruz Biotechnology Inc., CA, USA
LH receptor	Rabbit	Polyclonal, 1:1000	Human	Prof. Craig S. Atwood, William S. Middleton Memorial Veterans Hospital, Madison, USA
StAR	Rabbit	Polyclonal, 1:1000	Human	Santacruz Biotechnology Inc., CA, USA
AR	Rabbit	Polyclonal, 1:1000	Human	Dr. Elizabeth M. Wilson, University of North Carolina, USA
Resistin	Rabbit	Polyclonal, 1:500/1:4000	Mice	Dr. Nasreen Z. Ehteshaam, Inflammation Biology and Cell Signaling Laboratory, National Institute of Pathology, Safdarjung Hospital Campus, New Delhi 110029, India
β-Actin	Mouse	Monoclonal, 1:1000	Human	Santacruz Biotechnology Inc., CA, USA

morbidly obese humans compared with lean controls [22,23]. In past years, physiological role of resistin has been the subject of extensive study regarding its involvement with obesity and type II diabetes, inflammation and cellular stress [19–21,24–28]. Very few studies so far have investigated the involvement of resistin in regulation of female reproductive processes. Munir et al. [29] reported that the high circulating resistin play a role in causing ovarian hyperandrogenism in PCOS women .In bovine, resistin has been shown to be widely expressed in small and large follicles, corpus luteum, oocyte, cumulus, theca and granulosa cells (GC) while in rat resistin expression was present in corpus luteum, oocyte, theca cells, and weakly present in GC [30]. The localization of resistin has been demonstrated in the ovary of bovine and rat by Maillard et al. [30] and porcine by Rak-Mardyla et al. [31] but the role of resistin on ovarian function are conflicting and depends upon animal species, doses of resistin, and ovarian cell type used.

Therefore, primary aim of this study was to evaluate the relationship between adiposity and ovarian resistin expression and effects of resistin on ovarian activities of *S. heathi*. To achieve this specific aim, this study evaluated the following: (1) the seasonal variation in ovarian resistin expression in a natural population of the female bat, *S. heathi* and its relationship with changes in body fat mass and serum insulin, luteinizing hormone (LH), leptin, adiponectin and androstenedione levels during different reproductive phases; (2) localization of resistin protein in the ovary during different reproductive phases, and (3) *in vitro* effects of resistin on ovarian steroidogenesis with its particular relationship with synthesis and action of androgen on the ovary of *S. heathi*.

2. Materials and methods

2.1. Animals

The experiments conducted in present study were approved by the Institutional animal ethical committee of Banaras Hindu University, Varanasi, India. Details of the study site and feeding activity are described earlier in detail [32]. In Varanasi, the cold season lasts from November to mid February (mean ambient temperature < 20 °C) and dry season from March to June (mean ambient temperature > 30 °C). The bat, *S. heathi*, has a well-defined annual reproductive cycle at Varanasi, India. It attains sexual maturity within 3–4 months of their birth in July. All nulliparous bats (light yellow pelage on abdomen as compared with dark yellow pelage in parous bat) collected for this study were sexually mature (weighing above 30 g and wing-span exceeding 42 cm) and about 5 months old [33].

2.2. In vitro study

The *in vitro* study was performed on the ovaries of *S. heathi* ($n = 48$) collected during the month of February to determine the effect of resistin alone or along with LH on ovarian steroid production i.e., progesterone (P4), androstenedione (A4) and estradiol (E2), as well as expression of luteinizing hormone receptor (LH-R), steroidogenic acute regulatory protein (StAR), androgen receptor (AR), and insulin receptor (IR). Female *S. heathi* were sacrificed by decapitation as soon as they were brought to the laboratory. Ovaries were quickly taken out and cleaned for any adhered fat tissue and oviduct in medium Dulbecco Modified Eagle's Medium (DMEM; Himedia, Mumbai, India) containing 250 U/ml penicillin and 250 μg/ml streptomycin sulfate. Ovaries were cultured as described previously [15]. Culture medium was a mixture of DMEM and Ham's F-12 (1:1; v:v) (Himedia, Mumbai, India) containing 100 U/ml penicillin, 100 μg/ml streptomycin and 0.1%BSA (Sigma–Aldrich, St. Louis, USA). After initial incubation for 2 h at 37 °C, culture medium was discarded and ovaries (one per tube)

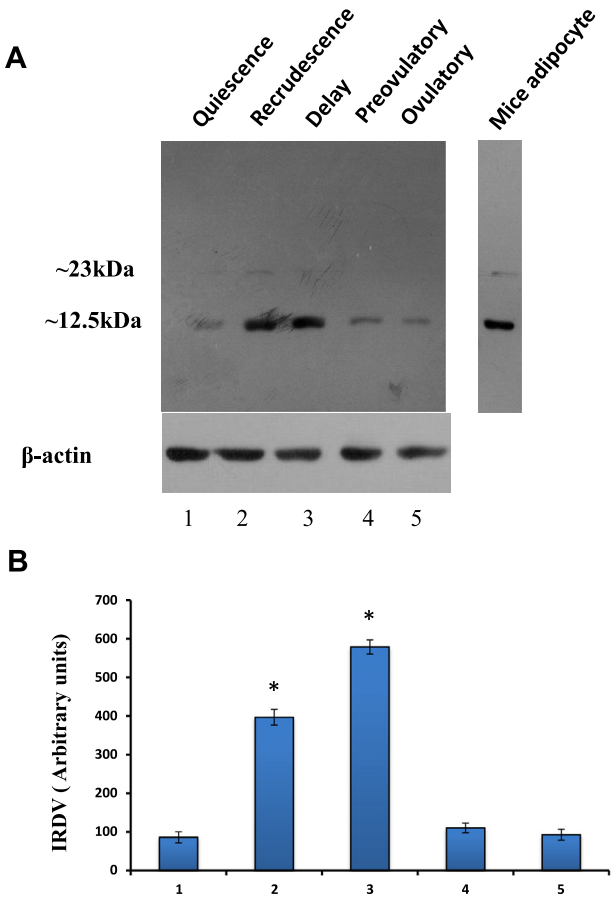

Fig. 1. (A) Western blot analysis of resistin protein in the ovary of *S. heathi* during the different phases of reproductive cycle. (B) Densitometric analysis of western blot of resistin protein in the ovary of *S. heathi* during different phases of reproductive cycle. Mice adipocyte was used as a positive control for resistin validation. Each sample was pooled from 2 to 3 bats and experiment was repeated thrice. Values are mean ± S.E.M.; $n = 45$. *Values are significantly ($P < 0.05$) different from other groups. IRDV = integrated relative density value.