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Applications of inhibitory intra-ovarian factor(s) in reproductive biotechniques to augment fertility in ruminants

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ABSTRACT

In the past, it was postulated that it is the extra follicular factors that have an important role on ovarian physiology and/or follicle growth. Later on, experiments revolving around dominant and subordinate follicles established the importance of intrafollicular factors on follicle growth. However, further experiments Led to the hypothesis that there exists intra follicular factors present in antral as well as preantral follicles that have role in follicle growth and intra follicular factors which are not necessarily stimulatory but also inhibitory in nature with respect to follicle growth. Studies indicated apart from stimulatory peptides, folliculogenesis was also modulated by inhibitory factors like oocyte maturation inhibitor, inhibin, FSH suppressing factor, follicle regulatory protein, FSH binding inhibitor, fibronectin inhibitory protein, aromatase inhibitor, inhibin binding protein, anti-mullerian hormone. The mechanisms where by locally produced bioactive molecules (intraovarian factors) regulate the ovarian follicular development and atresia is now the focus of research. We characterized ovine follicular fluid, identified and characterize a bioactive molecule in the ovary and demonstrated the physiological roles in follicular and oocyte development or atresia. Our study demonstrated the identification in follicular fluid of a novel 30.1 kDa peptide with non-species specific FSH-suppressing activity. Our results may be useful for the development of reproductive technologies to augment reproduction in mammals. The study may also contribute to understand the substrates to which oocytes and follicular somatic cells are exposed to in vivo in follicular fluid and may improve the culture conditions of oocytes and follicular somatic cells. ! 2013 Trade Science Inc. - INDIA

KEYWORDS

Inhibitory ovarian peptides;
Biotechnology;
Reproduction.

INTRODUCTION

Sheep are considered to be one of the excellent models for development of reproductive biotechnologies in ruminants and has been extensively used in basic and applied research. Though national wealth of sheep is worth Rs. 2400 crores and it contributes 20-30 billion GNP, the low reproductive efficiency of all indigenous sheep breeds except Garole is a stumbling block for converting the immense bio-resources into more remunerative economic wealth on sustainable basis to cope up with the rising demand of end –users. Efforts

are being directed to augment ovine reproductive efficiency by developing novel ovarian peptide for fertility regulation and by genetic improvement. Information on ovine follicular fluid proteins and their specific roles on the development of follicles, follicular somatic cells and oocytes are not available. Thus, the research problem was identified to isolate, purify and functional characterize a novel ovine follicular fluid protein.

BACKGROUND

Sheep breeds vary substantially in their physiological characteristics including reproductive traits, i.e ovulation rate, fecundity and lambing. A wide range of ovulation rate and litter size has been reported among and within different sheep breeds[1]. Majority of the Indian sheep breeds are suffered from low ovulation and fertility rates and single lambing[2]. The regulatory roles of intraovarian factors in ovine ovarian physiology are poorly understood. Ovarian follicular development and ovulation rate are determined by highly co-ordinated level of endocrine and paracrine signaling. The endocrine signaling or regulation implies role of ovaries, hypothalamus and pituitary glands while paracrine regulation involves signaling within follicular cells-oocyte, cumulus, granulosa and thecal cells[3]. Adopting a strategy that can maximize ovulation rate may enhance the litter size. Physiologist and geneticists with different approach are working on identification of key genes and physiological regulation that determines ovulation rates in sheep[4]. Mutation in the bone morphogenic protein receptor-1B (as in Booroola breed), growth differentiation factor-9, bone morphogenic protein-15 (BMP-15) and anti-Mullerian hormone result in an increased number of ovulations in sheep[5]. Augmentation of ovulation rates in Indian sheep breeds can be achieved by application of exogenous hormones[6], immunization of inhibin peptides[7] and introgression of FecB gene into non-prolific breeds[8]. Efforts are now being directed to improve reproductive performances by developing novel therapeutic agents for fertility regulation. It is necessary to understand the basic physiology underlying the complex process of folliculogenesis to address common causes of lower fertility and to devise innovative strategies to increase the efficiency of assisted reproduction technologies. The present study was undertaken with a goal to have a better understanding of ovine ovarian physiology and increased reproductive efficiency in sheep.

Basic mechanisms in folliculogenesis must be elucidated to reverse the downward trend in fertility in farm animals. Most ovarian follicles (99.9%) never reach ovulatory status, but undergo atresia at some point of developmental pathway[9]. Despite recent progress, the precise mechanisms underlying the follicular growth have yet to be fully elucidated. Though current knowledge of ovarian peptides is restricted to a few families (IGF-binding proteins, inhibin/activin/follistatin, and proteoglycans), the large array of peptides present in follicular fluid has to be characterized[10]. Unequivocal identification of these peptides and the factors that regulate their function will undoubtedly lead to a better understanding of the mechanisms controlling ovarian function. Follicular fluid peptides were reported to regulate the folliculogenesis and oocyte development both in homologous and heterologous species[11,12]. Other than the inhibin, activin and follistatin[13], other ovarian peptides viz., oocyte maturation inhibitor[14], granulosa cell inhibitory factor[15], a non-steroidal regulator of folliculogenesis[16], and oocyte maturation stimulator[17] were isolated from follicular fluid of various domestic animals, which regulate the ovarian functions *in vivo* and *in vitro* conditions. It may be possible that ovine follicular fluid peptides regulate the folliculogenesis and oocyte development in other species like buffaloes, the principle dairy animals in our country and seasonal breeders like ewes.

During follicular development, the follicular fluid constitutes the microenvironment for the oocyte. It contains substances implicated in oocyte meiosis, rupture of the follicular wall (ovulation), differentiation of the ovarian cells into the functional corpus luteum and finally fertilization[18]. Thus, the composition of follicular fluid reflects stages of oocyte development and the degree of follicle maturation. In consequence, certain components of follicular fluid might be of use as indicators for the maturation and thus the quality of the follicle[19]. Earlier studies had investigated peptides in follicular fluid[14,15,17,20], but only a few so far have described the protein pattern in follicular fluid and none the peptide pattern in follicular fluid in sheep.

STANDARD METHODS, PROCEDURES USED IN THE EXPERIMENTAL WORK

The present study was undertaken to examine the peptide profiles in ovine ovarian follicular fluid, to isolate of peptide(s) from follicular fluid and to investigate the

bioassays *in vivo* and *in vitro*. The follicular fluids aspirated from different size classes of ovarian follicles of slaughterhouse-derived sheep (*Ovis aries*) ovaries were made cell free by centrifugation (5000 g for 30 min). The biochemical analysis of follicular fluid was conducted by chemical method and auto-analyzer and the protein profiles of follicular fluid were examined by SDS-PAGE. The follicular fluid was made steroid free by charcoal treatment. The follicular fluid was then subjected to ammonium sulphate precipitation (0-20%, 20-35%, 35-50%, 50-65%, 65-75%, 75-85% and 85-95%) and then gel filtration chromatography using G-75 Sephadex. Peptide detection was performed by UV spectrophotometer at 280 nm. The 35-50% fraction yielded a detectable peak and a peptide of 30.1 kDa as examined by SDS-PAGE. The effect of increasing doses of the isolated ovine follicular fluid peptide was tested on ovarian responses and other organ weights in normal and superovulated rats (*Rattus norvegicus*). The peptide at different doses was also examined on preantral and antral follicle growth; cumulus cell expansion; oocyte maturation; biochemical changes in matured oocytes; mural granulosa cell, polar granulosa cell (cumulus cell), oviductal epithelial cell monolayer formation *in vitro* in sheep (*Ovis aries*, homologous species) and buffalo (*Bubalus bubalis*, heterologous species).

PRINCIPAL RESULTS OBTAINED

1 A NOVEL single-chain peptide of 30.1 kDa molecular weight having FSH suppressing activity was isolated from ovine ovarian follicular fluid[21].
2 The peptide had an inhibitory effect on antral follicle and oocyte growth *in vivo* in normal and superovulated rats[22].
3 The peptide inhibited oocyte, cumulus and granulosa cell, antral follicle growth *in vitro* but without any significant effect on oviductal cell (extra ovarian cell) and ovarian preantral follicle growth *in vitro* in buffalo[23].
4 Metabolite, electrolyte, and enzymatic composition, for the first time, of ovine follicular fluid were determined[24].

TECHNOLOGICAL RELEVANCE

A good understanding of the processes involved in the growth and differentiation of ovarian follicles destined for ovulation is essential in order to optimize sheep reproduction[25]. Greater emphasis has been placed by researchers on the roles of extra ovarian regulators (for example, pituitary gonadotropins) than on intraovarian factors for follicular growth. The mechanisms whereby locally produced bioactive molecules (intraovarian factors) regulate the ovarian follicular development and atresia is now the focus of research[26]. The present study intends to biochemical characterize ovine follicular fluid, to identify and characterize bioactive molecule in the ovary and demonstrate the physiological roles in follicular and oocyte development or atresia. Moreover, the results may be useful for the development of reproductive technologies to augment reproduction in mammals. The study may also contribute to understand the substrates to which oocytes and follicular somatic cells are exposed to *in vivo* in follicular fluid and may improve the culture conditions of oocytes and follicular somatic cells in sheep.

For example: This study demonstrated the identification in follicular fluid of a novel 30.1 kDa peptide with non-species specific FSH-suppressing activity.

Uses of the isolated peptide could be:

(a) Suppressing or raising FSH levels in a mammal
(b) Increasing or suppressing the ovulation rate of a mammal
(c) Augmenting or reducing fertility in female mammal
(d) Advancing or delaying the onset of puberty in sexually immature mammal
(e) Determination of the fertility status of a mammal
(f) Each of these methods comprising administration of the peptide or its antibody to the mammal at appropriate dosage.

SOCIOECONOMIC RELEVANCE

India possesses 75 million sheep and rank third in the world in sheep population. The spectrum of sheep breed diversity is most pronounced in India with 42 distinguished breeds well adapted to specific environment and with sustainable producing potential in specific agro-climatic regions[27]. However, inherent problems like delayed maturity, seasonality of breeding, follicular atresia, embryonic mortality susceptibility to thermal stress and infertility are the major constraints in the reproductive performances and the productivity of this important species[27,28]. It is necessary to understand the basic physiol-

ogy underlying the complex process of folliculogenesis to address common causes of lower fertility and to devise innovative strategies to increase the efficiency of assisted reproduction technologies. Sheep represents a valuable model system for the elucidation of the endocrine and local mechanisms controlling follicle development[5,29]. The results of the investigations may have direct strategic relevance in clinical medicine in farm animals and development of strategies to increase the efficiency of assisted reproduction technologies.

Sheep rearing continues to be a backward profession, primarily in the hands of poor, landless or small and marginal farmers who own either an uneconomical holding or no land at all. Unlike exotic breeds of sheep (e.g. Boroola, Romney or Merino), which have high fecundity, the Indian breeds of sheep, is marked by an ovulation rate of one and a low incidence of twinning[7]. It is anticipated that the present study will underpin the development of improved methods for manipulating reproduction and for diagnosing and treating infertility in sheep, and possibly other farm animals.

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