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Genomics Control of Folliculogenesis in Animals with Emphasis to Buffaloes

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Abstract: Reproductive efficiency is high priority in all breeding systems and required more critical studies for its understanding especially in poor and seasonal breeder subjects.Geneticsis the main pillar for the selection of genetically superior animals that can pass their desired traits to their offspring. Molecular biology has led to the generation of techniques and knowledge that assist and complement the traditional system of genetic improvement. Recently, there is a progress in the elucidation of factors regulating oocyte and follicle growth and development, as well as oocyte maturation, through the study of the crucial roles of a large number of proteins expressed throughout oogenesis, in particular during the early stages of folliculogenesis . The present article skimmed most of recent studies carried on the role of genomics to control the animal fertility and enumerates some fertility markers that may help to improve the reproductive performance of cows and buffaloes, especially the later that. Light was thrown on roles of transforming growth factors(TGF), gonadotropins (FSH and LH),steroids, Cytochrome p450 controls sex steroid synthesis and metabolism,Insulin growth factor (IGF)and Leptin. It was concluded that more researches are required to understand this mechanism, especially in poor and/ or seasonal breeders such as buffaloes in order to ameliorate their productive potentials.

Key words: Genomics · Folliculogenesis · Animals · Buffaloes

INTRODUCTION

Reproductive efficiency is high priority in all breeding systems, especially in seasonal breeders as the opportunity for mating and getting the female pregnant is time limited [1]. Consequently, one of the great challenges of the underling biologists is to gain an understanding of the underlying biology of the buffalo cow that contributes to low fertility and develop strategies to improve fertility. The breeding program is one of the most important technologies used in the production process, which consider genetics the main pillar or instrument when it comes to the selection of genetically superior animals that can pass their desired traits to their offspring. This breed is used extensively in many countriesdue to its great potential for production, rusticity and adaptation to various environmental conditions [2].

Currently, research in molecular biology has led to the generation of techniques and knowledge that assist and complement the traditional system of genetic improvement, intensifying research on the occurrence of different types of molecular markers in the bovine genome, in order to provide more information to assist studies on the quantitative characteristics of zootechnical interest [3]. Recently, molecular genetics has shown great advances, mainly due to the development of methods for the analysis of genetic material using a large number of samples, with consequent cost reduction [4]. Also, there is a progress in the elucidation of factors regulating oocyte and follicle growth and development, as well as oocyte maturation, through the study of the crucial roles of a large number of proteins expressed throughout oogenesis, in particular during the early stages of folliculogenesis [5].

The present article skimmed most of recent studies carried on the role of genomics to control the animal fertility and enumerates some fertility markers that may help to improve the reproductive performance of cows and buffaloes, especially the later that has been traditionally regarded as a poor breeder [1].

In the last decade, a substantial progress has been made in the elucidationof factors regulating oocyte and follicular growth and development,as well as oocyte maturation, through the study of the crucial roles of a large number of proteins expressed throughout oogenesis, in particular during the early stages of

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folliculogenesis. This has mainly been accomplished by assessing the lack of their gene products either by using knockout approaches and/or targeted deletion.

Transforming Growth Factors (TGF) Superfamily and Other Proteins: Oocytes originate from primordial germ cells (PGCs) and their development initially depends on signals derived from both the extra-embryonic ectoderm and visceral endoderm. Members of the TGFβ family such as bone morphogenetic proteins (BMPs), BMP4, BMP8b (Ectoderm origin) and BMP2 (Endoderm origin) are specific factors needed for PGC formation and regulation of gene expression [6-8].

PGCs migrate to the genital ridge where they proliferate by mitosis and give rise to oogonia. Migration, proliferation and colonization of PGCs to the developing gonads are controlled by many factors and depend as well on the interaction of PGCs and their surrounding somatic cells. Many oocytes that are not surrounded by somatic cells undergo apoptosis [9].

As soon as PGCs are formed, the initially bipotential gonad will continue its differentiation mostly under the influence of somatic cell derived transcription factors GATA4, FOXL2, LHX9,WT1,WNT4 and SF1 [10]. After colonization of the gonad (~E13.5), PGCs will undergo a phase of mitotic proliferation with an incomplete cytokinesis, leading to the formation of 'Germ cell cysts' or 'Germ cell nests' [11]. Following this event and before follicle formation mitotic divisions stop and germ cells initiate meiosis, become primary oocytes and commit to the female program of development [12].

STRA8 (Stimulated by retinoic acid gene 8) is a cytoplasmic factor expressed by female germ cells just prior to entering the prophase of first meiotic division [13] and in response to retinoic acid (RA) [14-16]. In females, STRA8 is required for premeiotic DNA replication as well as for meiotic prophase events (i.e.chromosome condensation, cohesion, synapsis and recombination) [17]. Oocytes remain at stage of meiosis throughout oogenesis, until LH induces final oocyte maturation (In most mammals). Around the time of meiotic arrest, germ cell nests breakdown to initiate follicle formation. Oocytes become surrounded by somatic (Pre-granulosa) cells and form primordial follicles [11].

The processes of germ cell cyst maintenance and breakdown are not fully understood. Some studies point out to a role of estrogens in the maintenance of the germ cell nests [18-20] via the estrogen receptor (ER)-β [21]. Besides steroids, members of the transforming growth factor beta (TGF-β) superfamily (Such as GDF9 and BMP15) and other proteins such as FOXL2 and NOBOX also seem to be involved in this process[22]. Lack of these genes or a reduced expression and function of the gene products affect the timing of nest breakdown and impair this process [23,24]. Many pro- and anti-apoptotic proteins have been demonstrated to regulate germ cell death. For instance, in the absence of BCL2, an anti-apoptotic member of the B cell lymphoma/leukemia (BCL) protein family, a reduced number of oocytes and primordial follicles, but a normal number of primary follicles have been reported at an early age (6 weeks), suggesting that BCL2 may have an impact on follicle survival during the establishment of PGC and/or primordial follicle formation. A similar role has been suggested for BCLX [25]. On the other side, the pro-apoptotic protein BAX, also member of the BCL family, promotes cell death. It has been reported that loss of Bax or loss of its regulator Ahr(Aryl hydrocarbon receptor), results in increased number of primordial follicles as observed at 6 weeks postnatal [26].

Other important growth factors such as neurotrophins (Which mainly play a role as part of the nervous system); have also been shown to regulate early folliculogenesis. For instance, nerve growth factor (Ngf) is expressed both in somatic cells and oocytes even prior to follicle formation and seems to play a role in primordial follicle activation [27].

Granulosa cell expression of anti-Mullerian hormone (AMH), a member of the TGFβ superfamily, is required to maintain a balance between the number of primordial follicles being activated and those that remain stay in the resting pool [28,29]. Primordial follicles will give rise to primary follicles in which the flattened granulosa cells will develop into single-layered cuboidal granulosa cells. The early expression of two other transcription factors from oocyte origin, Sohlh1 and Nobox, is decisive for the progression of primordial follicles to the next primary follicular stage. Progression from the primary to the secondary follicular stagecan take place in the absence of hormones,although follicle stimulating hormone receptors (FSHR) are present [30].

Activins and inhibins, two closely related proteins with opposite roles belong to the TGFβsuperfamily and also influence follicle development.

Both protein complexes consist of two subunits that originate from the same family of related genes and proteins, but differ in their composition [31]. Alongside an increase of follicular diameter and granulosa cell differentiation, the activin tonus decreases whereas the inhibin expression increases till the follicle differentiate to become an antral follicle[32,33].

Role of Gonadotropins Hormones, Follicle-stimulating Hormone (FSH) and Luteinizing Hormone (LH): When follicles reach the preantral stages, development throughout this period and progression to the early antral stage still rely primarily on intraovarian factors; however, unlike in earlier stages, follicles express functional FSH and LH receptors and are able to respond to gonadotropins. *In vitro* studies have shown that cultured preantral follicles respond to gonadotropins [34] and exposure of early preantral follicles to FSH is favorable for follicle survival and required for *in vitro* antrum formation to occur [35,36].

FSH starts and maintains follicular development by binding to its specific receptor (FSHR) in the surface of the granulosa cells in the ovary [37]. This binding allows the activation of the FSHR codifying gene [38]. Granulosa cells within preantral follicles proliferate at a very high rate, giving rise to a multi-layer preantral follicle, an increase in follicular size, followed by the appearance of an antral cavity. The progression throughout the antral stages and ovulation has been considered to be dependent on pituitary-secreted gonadotropin (FSH and LH) support.

FSH is the essential driver of *in vivo* antral development. FSH induces luteinizing hormone receptor (Lhcgr) mRNA expression in mural cells, which will be required for follicles to respond to LH, the latter being crucial for triggering the ovulatory process. Action of both gonadotropins in the ovary is mediated by binding and activation of their receptors (LH receptor, LHR and FSH receptor, FSHR). Knockout mouse models of each of these receptors have shown the relevant role of gonadotropin signaling within the ovary.In LHR knockout (LuRKO) mice, follicle development does not progress beyond the antral stage; these mice are infertile due to the low estrogen production and anovulation [39]. Moreover, high doses of FSH are not capable of inducing final follicular development and ovulation when LHR is not present [40]. Therefore, expression of LHR is essential not only for ovulation, but also for follicle maturation prior to ovulation.

LH is a glycoprotein hormone of pituitary origin that regulates gonadal function, including steroidogenesis and gametogenesis [41]. The identification of differential signaling pathways that regulate LHR gene expression is of major relevance to the understanding of normal reproductive physiology and the pathology of reproductive disorders [42]. The sequence alignment of Egyptian buffalo LHR with published sequence (accession numberD Q 8581701,Bubolus bubalis) was carried out using BLAST and showed that the Egyptian buffalo LHR possess identities at 98% with five SNPs; (T/C) at position 34, (G/A) at position 60, (A/G) at position 61, (T/C) at position 88 and (A/T) at position 285 in the reverse-primer region [43].

The existence of allelic variants in FSHR gene was reported in cattle [44,45] causing desensitization of the FSH receptors in the cell membrane which results in a less efficient hormone signal [46]. One SNP (G/A) at position 59 was traced in Egyptian buffalo FSHR gene caused desensitization of cell receptors and less efficient hormone signal transmission [43]. On the other side, when one SNP of G278A, located in the 5' upstream region of bovine FSHRgene, was found in Chinese Holstein cows with CC genotype, a significant increase in the total number of ova and more transferable embryos was collected than those of the CD and DD genotypes [47].

The Influence of Steroids: Follicles synthesize steroidhormones such as androgens and estrogens, which contribute to follicular development, by inducing granulosa cell proliferation and differentiation via the androgen receptor (AR) and estrogen receptor(ER), respectively [48]. Through the well-known two-cell,two-gonadotropin model, theca cells produce androgens under influenceof LH stimuli, whereas granulosa cells produce estrogens usingandrogens as a substrate, under influence of FSH [49].

Androgens, have been shown to participate in granulosa cell proliferation and survival via the androgen receptor (AR). AR-deficientfemale mice are subfertile and show a reduced number of antral folliclesand ovulated oocytes and a high rate of granulosa cells apoptosis;they eventually develop premature ovarian failure [50,51].

In preovulatory follicles, a critical role of GDF9 and BMP15 in the induction of cumulus mucification/expansion and the regulation of genes involved in this process has been demonstrated [52,53]. Other factors such as theca-derived factors BMP4 and BMP7 are potential paracrine regulators of granulosa cell function. In the rat, these two factors attenuate FSH-induced progesterone secretion whilst they enhance FSH-induced estradiol secretion [54].

The estrogen receptor-α (ERα) and its gene is considered a candidate marker for reproduction and functional traits in farm animals [55].A polymorphism within the 5’ region of the bovine ERgene (A/G transition) was identified. Also a Study was carried out on Egyptian buffalo showed that the sequence alignment of ERαcorresponding to published sequence (Accession number. AY340597.1, Bison bonasus) using BLAST showed that G-allele possess identities at 99% with 3 SNPs; one SNP (G/A) at position 89 and 2 SNPs (A/G) at positions 200 and 201 and A-allele possess identities at

98% with these 3 SNPs found in allele G in addition to another SNP (A/G) at positions 165.18 out of 100 investigated Egyptian buffaloes in this study (18%) are genotyped as AG with three digested fragments at 248-, 171- and 77-bp and the remaining 82 animals (82%) are genotyped as GG with two digested fragments at 171- and 77-bp [43]

Cytochrome P450 Controls Sex Steroid Synthesis and Metabolism: During sexual differentiation of the genital ridge in early embryogenesis, the transcription factor steroid-factor-1, a member of the nuclear hormone receptor gene family, is pivotal in upregulation of P450 genes implicated in steroid hormone synthesis-including members of the CYP11, CYP17, CYP19 families [56]. CYP11A1is mitochondrial enzymes controlling the production of progesterone.CYP17A1 is needed for synthesis of cortisol, testosterone and oestrogen, whereasCYP19A1 converts androgenic precursors into estrogens. Both CYP17A1 and CYP19A1 are locatedwithin the endoplasmic reticulum.CYP17A1 is a dual-function enzyme, catalyzing 17α-hydroxylation of steroid substrates and cleavage andoxidation of their side-chains. Mutations in CYP17A1that impair both these enzymic activities lead to deficiencies in production of glucocorticoids and sexsteroids, whereas those that prevent oxidation and shortening of the side-chain lead to deficiencies in sex steroids only [57]. CYP19A1 synthesis estrogen by aromatisation of theA ring of the androgenic steroid substrates. Loss-offunctionmutations in CYP19A1 cause androgen excess,which leads to improper virilisation in females and hypervirilisation in males of function mutations in CYP19A1 produce gynaecomastiain males [58]. The CYP19 gene has been mapped to band q2.6 on chromosome 10 and q2.6 on chromosome 11 in cattle [59,60] and buffalo [61], respectively. The gene size ranges from 56 kb to 120 kb in different species. It consists of 10 exons. Though transcripts of CYP19 in adipose tissue, skin, ovary and placenta have different 50UTRs the coding region and thus the aromatase protein expressed in various tissue sites is always the same regardless of the promoter used. Hence polymorphic analysis of the coding region is very important in addition to the study of tissue specific regulation of CYP19 gene. It was found that mice deficient in aromatase (ArKO) due to targeted disruption of the CYP19 gene displayed under developed external genitalia and uteri, ovaries having numerous follicles that are arrested before ovulation, no corpora lutea, atretic follicles and prepubertal mammary gland development. The mutations in CYP19 gene cause aromatase deficiency that result in sexual ambiguity with clitoromegaly,multicystic ovaries, pseudohermaphroditism, progressive signs of virilization, tall stature and pubertal failure with no signs of estrogen action in females[62]. It was proposed that the gene polymorphism in CYP19 influence the fertility of cattle [60] and buffaloes. The SSCP and sequence analysis revealed 4allelic variants in coding exons and introns which unaltered the protein sequence. However, a significant polymorphism (T/C heterozygote) was found near TATA binding protein region in regulatory part (A facet of promoter II) at position 23 of CYP19 exon 2, in all late matured buffalo caws [63].

In general, in rats, humans and ruminants, the expression of CYP19 mRNA is primarily stimulated by follicle-stimulating hormone (FSH) [64]. Detailed studies on its regulation in humansFSH alone had no effect on this signaling molecule but produced synergistic response in Akt phosphorylation in the presence of IGF1. Therefore, similar molecules were recruited by FSH and IGF1 functioning through same signaling pathway resulting into enhanced gene expression and hormone production.

Insulin Growth Factor (IGF): During the last decade, the role of growth factors in ovarian folliculogenesis has been extensively studied in mammals. In particular, a growing body of evidence indicates that the insulin-like growth factor (IGF) system plays a key role in follicular development and atresia in the woman, rodents and domestic animal species.

The IGF System Is Composed of Different Elements [65]

- Two ligands, IGF-I and -II.
- Two receptors; the type I receptor mediates most of the somatomedin-like actions of both IGF-I and -II. It is structurally and functionally related to the insulin receptor.The affinity of this receptor for IGF-I is slightly higher than for IGF-II and much higherthan for insulin. The type II receptor binds IGF-II. This receptor does not bind insulin and binds IGF-I with verylow affinity.
- Six IGF binding proteins (IGFBPs), which bind IGF-I and -II with high affinity [66] are present in all biological fluids.IGF-I stimulates both proliferation of granulose cells (Small follicles from 1to 3mm diameter) and differentiation (>5mm diameter) to stimulategranulosa cells to secreat progesterone [67]. Also, stimulates steroidogenesis in thecal cells [68,69].

Pig and rodents have IGF-I mRNA localized with FSH receptor mRNA in granulosa of healthy growing follicles, whereas IGF-II mRNA is expressed in granulosa of healthy as well as atretic follicles [70] while, ovine and bovine ovaries do not appear to express significant levels of IGF-I [71]. Rather, IGF-II expression has been observedin thecal cell of both species, its expression being higher in small "gonadotropin-independent" than in large "gonadotropin-dependent" follicles [72,73] The intrinsic capacity of granulosa cells to respond to IGF-I stimulation may change during growth of the follicle. *In vitro* studies showed that percentage of proliferating cells was about 70% in granulosa cells from small follicles and less than 10% in cells from large follicles [74]. This difference in proliferation rate might be associated with a change in intracellular signalling pathway during follicular growth. Forexample, c-jun and c-myc protein are selectively expressed in mitotically active, not in differentiating granulose cells in the rat [75] and human [76]. So it can then be hypothesized that high levels of intracellular factors like c-jun and c-myc may promote proliferation rather than differentiation of granulose cells in response to IGF1. It is hypothesized that the increase in expression of IGF-I in large preantral follicles results in an increase in the number of functional FSH receptors, leading to an increase in IGF1receptors as shown in *vitro* and *vivo* in rats and cattle. This positive feedback loop might partly be responsible for the amplification of FSH action, the formation of the antrum,the expression of aromatase and LH receptors in fully mature follicles.In cow, GH treatment led to marked increase in IGF-I levels in serum as well as numberof 2-5mm healthy growing follicles.In the same time it did not affect the number of ovulatory follicles or the concentration of estradiol, progesterone, FSH and LH in serum [77].

The intrafollicular levels of IGFBP-2 and -4 strongly decrease by progressive growing of follicles (From 1 to 2mm diameter to preovulatory follicles) [72,78,79]. In contrast, intrafollicular levels of the same IGFBPs, as well as IGFBP-5 in the ruminants, strongly increasein atretic follicles. These changes are due to both changes in mRNA expression and changes in their proteolytic degradation. Intrafollicular cleavage of IGFBP-2, -4 and -5 might participate to the increase in bioavailability of IGFs that further stimulate granulosa cell proliferation and steroidogenesis [79].A recent study on follicular fluid in infertile buffalo cows showed that acyclic buffaloes have lower concentrations of estradiol and insulin concurrent with higher concentrations of progesterone in the follicular fluid. These hormonal changes in the follicular microenvironment are possibly a manifestation of the disturbances in the normal follicular development leading to anovulation and anestrus in acyclic buffaloes.

Leptin Hormone: The ovarian cycle of the mammals is characterized by repeated patterns of cellular proliferation, differentiation and transformation which is followed by follicular development and formation and regression of the corpus luteum.

Leptin, a 16.4 kDa peptide hormone, product of the obese gene, is secreted primarily in adipocytes and is known to play a critical role in the regulation of body weight by inhibiting food intake and stimulating energy expenditure [80]. Apart from its role in the regulation of body weight and energy expenditure, evidences suggest direct involvement of leptin in ovarian function in a majority of animal species [81,82]. Its role in reproduction includes important actions on the hypothalamus to bring about release of LH-releasing hormone, thereby triggering gonadotropin release and leading to development of the reproductive tract and induction of puberty [83]. IGF-1 has direct stimulatoryeffects on key components of the steriodogenic pathway to increase progesterone secretion in bovine luteal cells. In particular, IGF-1 is able to induce the expression of StAR mRNA in bovine granulosa-derived luteal cells [84]. The stimulatory effects of leptin on porcine GC steriodogenic function have also been attributed to the induction of StAR transcription, as the result of sterol regulatory element binding protein 1(SREBP1) modulation [85]. Therefore, it is feasible to suggest that it is the synergistic effects of leptin and IGF-1 on StAR transcription, which leads to a response great enough to increase progesterone synthesis [86]. Leptin receptors have been found in the hypothalamic center beside the widespread distribution in tissue, including liver, heart, kidney, lung, small intestine, testes, ovaries, spleen, pancreas and adipose tissues in human, mouse,rat, pig and bovine ovaries [87,88]. Infertility in animals may be caused by a defective corpus luteum (CL), which can be attributed, in part, to incomplete vascularization (Angiogenesis) of the corpus luteum, causing a decrease in progesterone production. The angiogenic process is regulated by proangiogenic factors including vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1) and fibroblast growth factor 2 (FGF-2) that are expressed at different times during the luteal phase. Nevertheless, it is known that leptin induces angiogenesis [89,90].

In conclusion, recent investigations quietly potentiate evidences for genomics control of folliculogenesis in Animals. More researches are required to understand this mechanism, especially in poor and/ or seasonal breeders such as buffaloes in order to improve their productive performance.

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