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Polycystic Ovary Syndrome and Non-Classic Congenital  
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# Novel Systematics of Nomenclature and Classification of Female Functional Androgenization (Including Polycystic Ovary Syndrome and Non-Classic Congenital Adrenal Hyperplasia)

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**Objective:** A novel nomenclature as well as a comprehensive, clearly defined classification of functional androgenization (FA) from puberty well into postmenopause have been developed. Data are presented indicating the applicability of this algorithm. **Design:** Retrospective case-control study involving FA-patients, and controls (C). **Methods:** FA-patients were classified into five groups, functional cutaneous androgenization (FCA: skin) as well as functional androgenizing syndrome (FAS) I (ovary), II (adrenal), III (multi-organ-disorder with FA, obesity, hyperinsulinaemia) and IV (residual FA dysfunctions) using group-specific variable clusters. They are set up by primary (classifying) variables such as cutaneous androgenetic symptoms (acne vulgaris, hirsutism, androgenetic alopecia), body mass index (BMI), testosterone, free androgen index (FAI), polyfollicular ovaries (PFOs), and 1-h-insulin (after oral glucose loading). Groups FCA and FAS I–III were sub-classified through classic full-blown (“a”) and non-classic, minimum standard core/miscellaneous clusters (“b”). Variables were allocated as integral part of different clusters (e.g. enhanced BMI: in FCAb, FAS IIb, FAS IIIa/b, and FAS IV). Patients’ complete characterization was achieved additionally by using secondary (facultative) variables, e.g. triglycerid levels. **Results:** The FA-groups included 6, 33, 10, 59, and 18 subjects. All FCA-patients presented cutaneous androgenetic symptoms, PFOs were visualized in all FAS I and III patients. Group FAS Ia showed highest LH levels, and testosterone was higher in FAS I vs. FCA, FAS II, FAS IV and C. Levels of DHEAS were found to be highest in group FAS II. BMI and triglycerids were higher in FAS III vs. FCA, FAS I, FAS II, and C, and one-hr-insulin in FAS III was higher vs. FCA, FAS I, and C. In FAS IV covering the residual FA-patients, several obese, hyperinsulinaemic individuals were classified who showed an increased FAI without the presence of PFOs. Significant *P*-values were found to be between < 0.05 and < 0.0001. **Conclusion:** An essential paradigm shift in the diagnosis of androgenized females was presented using an exactly predefined classification of a manageable number of distinguishable FA-entities. An exactly repeatable diagnostic stratification is essential in order to guide customized treatment options by identifying patients’ individual dysfunctions and disorders and by improving their risk assessment. Such an approach may also improve the scientific methodology of clinical studies. **J Reproduktionsmed Endokrinol 2010; 7 (1): 6–26.**

**Key words:** androgenization, PCOS, PCOD, AGS, NC-CAH, hyperandrogenaemia, hyperinsulinaemia, hirsutism, alopecia, acne, obesity, metabolic syndrome, diabetes, classification

## ■ Introduction

“Female androgenization” is used to define a wide spectrum of heterogeneous dysfunctions and disorders comprising one or several organs or organ systems, and touching on paediatric and adult endocrinology, gynaecology, reproductive medicine, dermatology, as well as molecular genetics. It is an inbuilt difficulty for the clinician who takes care of androgenized patients to cover the entire range of androgenized phenotypes. An algorithm to stratify the diagnosis using a well defined classifying (grouping) of androgenized female individuals is clearly warranted but no such a unifying clinical and scientific approach is available so far.

Currently, the different terms and definitions referring to female androgenization do not follow any systematic ap-

proach (Tab. 1). The term, “idiopathic hirsutism” as an example [1–5] describes a cutaneous androgenetic symptom whereas rather a topic than a systemic phenomenon appears to be predominant, and so far, known, unknown or clinically non-identifiable, independent factors uniquely allocated in the skin might be responsible [6, 7]. In addition, the commonly used term “polycystic ovary syndrome/disease (PCOS/D)” is focused on the ovarian origin of functional androgenization (FA) [8–11]. Two major “PCOS/D” subsets, one without and one with the presence of the metabolic syndrome (MetS) are established by the Rotterdam consensus 2003 (RCO3) [12, 13]. Further terms of the “PCOS/D” such as “non-PCO PCOS” (hyperandrogenism without “PCO”) [14] or “non-hyperandrogenic PCOS” [15–17] are covered by the RCO3 definitions as well. Moreover, the adrenal part

of FA is defined through terms such as “congenital adrenal hyperplasia (CAH)” and “non-classical CAH (NC-CAH)” [18]; synonymous terms are: congenital “adrenogenital syndrome (AGS)”, and “late-onset AGS”, respectively [19]. Regarding CAH and NC-CAH, an expert conference has taken place as well [20] in order to generate universally reliable definitions of those disorders.

Statements of the RCO3 [12, 13] are regarded to be the current diagnostic standards of the PCOS/D. However, a lot of concern exists against the term itself and the proposed definitions. Long before the RCO3 criteria have been published [12, 13], the term “PCOS” has been declared as “meaningless in terms of diagnosis and may lead to considerable confusion” [21], and later on as a “misnomer” [22]. In addition, the content of the RCO3 [12, 13] has been subjected to a

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**Table 1:** Novel nomenclature and classification of functional androgenization vs. current equivalence terms

| Terms                  | Classification/grouping                    |                 | Equivalence terms currently used |                                                                                                          |
|------------------------|--------------------------------------------|-----------------|----------------------------------|----------------------------------------------------------------------------------------------------------|
|                        | Organ, organ system predominantly involved | Groups, subsets |                                  |                                                                                                          |
|                        |                                            | #               | terms                            |                                                                                                          |
| FCA                    | Skin                                       | 1               | a<br>b                           | E.g. idiopathic hirsutism [2]<br>Non-hyperandrogenaemic PCOS [12, 13, 30]<br>Asymptomatic PCOS [73, 135] |
| FAS                    | Ovary                                      | 2               | I a/b                            | PCOS/D; patients without metabolic syndrome [12, 13]                                                     |
|                        | Adrenal gland                              | 3               | II a/b                           | Cryptic AGS [19]<br>Symptomatic AGS [19]<br>Late onset AGS [19]<br>NC-CAH [18, 20]                       |
|                        | Ovary, fat tissue, pancreas, liver         | 4               | III a/b                          | PCOS/D; patients with metabolic syndrome [8, 12, 13]<br>HAIR-AN syndrome [163]                           |
|                        | –                                          | 5               | IV                               | Non-PCO PCOS [12, 13, 30]                                                                                |
| Differential diagnosis |                                            |                 |                                  | see Table 4                                                                                              |

FCA: functional cutaneous androgenization; FAS: functional androgenizing syndrome; PCOS/D: polycystic ovarian syndrome/disease; AGS: adrenogenital syndrome; NC-CAH: non-classical congenital adrenal hyperplasia; HAIR-AN: hyperandrogenaemia-insulin-resistance-acanthosis-nigricans syndrome.

critical statement [16], and a controversial debate [23, 24] whereupon the “Rotterdam criteria” have been termed “premature” [24]. Moreover a substantial critical analysis [25] has complained a simplification of the underlying complexity as well as an over-diagnosis of this ovarian disorder by the RCO3 criteria. Using these criteria an expansion of “PCOS/D” phenotypes has been noted [26] exemplified in a marked increase of the diagnosis “PCOS/D” in patients [15] with “oligo-ovulation” [27] as compared to the former NIH 1990 criteria [28]. In consequence, the reproducibility of the diagnosis “PCOS/D” is strongly questionable both under clinical and scientific conditions. Additional critical standpoints have been stated [29]. The task force of the Androgen Excess Society Guideline from 2006 (AESGO6) [30], and Azziz as the leading author [31] of the AESGO6 have proposed a modification of the NIH 1990 criteria

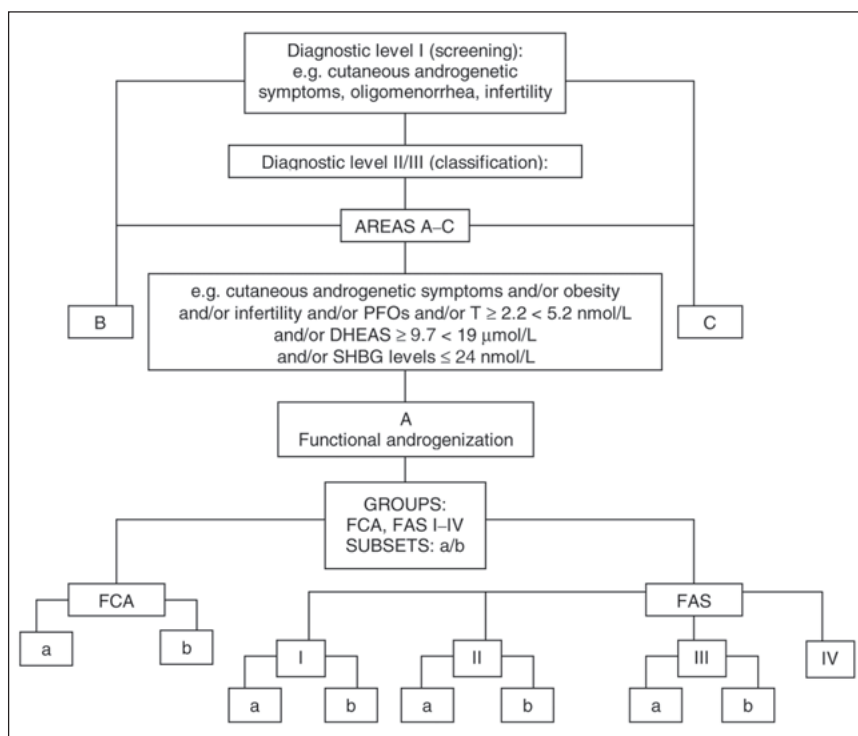
[28]: However, the AESGO6 criteria do not conquer the pitfalls of misdiagnosis, for example, because of combinations of symptoms (e.g. “hyperandrogenemia” and “oligoanovulation”: phenotype “D”) which fail to be appropriate in making the diagnosis “PCOS/D” in a considerable number of individuals [25].

In contrast to the comprehensive and critical comments on the RCO3 [25, 29] there is no comparable analysis published from the gynaecological point of view concerning NC-CAH.<sup>1</sup> Milder subgroups<sup>1</sup> of the CAH will not be uncovered by imaging methods like ultrasound or MRT scan. Thus, the term NC-CAH conveys a clinical symptomatology that usually is not part of the real phenotype behind the diagnosis in cases with pubertal onset of androgenizing symptoms. Similarly, the synonymous term “late onset AGS” [19] is, as a rule, misleading since the the ambiguous genitalia im-

plied by that term are never seen when symptoms of androgenization appear with or after puberty; the rarely observed clitoromegaly might be an exception. It is therefore evident that those two terms are further examples of “misnomers” in the field of female androgenization.

While recognizing that many diseases in gynaecology and obstetrics are classified in a sophisticated and individualized way (e.g. the risk categories of breast cancer) [37] the various kinds of female androgenization are still in urgent need of such a classification. Due to the high variability of androgenizing symptoms and the many factors impacting androgenization in female individuals this is not a simple task. In fact, almost every constellation and combination of variables one may think of are conceivable. Nevertheless, the present study attempts to set up a markedly modified and new systematic nomenclature, and, even more

<sup>1</sup> With regard to the especially interested reader these disorders are described more extensively in the following. Classic CAH is a family of monogenic, recessive autosomal disorders which are caused by a severe deficiency in adrenal steroidogenesis resulting in one of the most severe functional-endocrine disorders [32]. Around 90 % of the cases are caused from homozygous or compound heterozygous recessive mutations/deletions of the *CYP21A2*. Such gene defects consecutively lead to a deficient cortisol production due to a strongly reduced or completely missing activity of encoded CYP21A2 enzymes [18, 20, 32]. Diminished circulating cortisol levels signal the pituitary to enhance the secretion of adrenocorticotropin (ACTH), and its chronic hypersecretion results in the development of an adrenal hyperplasia. Finally, an excess of adrenal C<sup>19</sup>-sexual steroids is triggered. The latter dysfunction is the cause of prenatally induced congenital virilization of female newborns (ambiguous external genitalia) which might be reduced or eliminated when an adequate dexamethasone therapy of the mother starting at early pregnancy [33] has been performed. A complete substitution of glucocorticoids (simple virilising form) or of both gluco- and mineralocorticoids (salt wasting form) is required during the entire life beginning from the postnatal phase. In contrast, the milder form, the NC-CAH, does not show any symptoms at birth (except of a rarely found clitoromegaly). It is characterized, however, through a later onset (mostly at early infancy) of symptoms, particularly through precocious pubarche [34] with acceleration of bone aging and growth velocity in both sexes, and early menarche, clitoromegaly and severe cutaneous androgenetic symptoms in female individuals (if not diagnosed timely and adequately treated). The mildest distinctness of female phenotypes including a variety of symptoms such as cutaneous androgenization, menstrual disturbances, hyperandrogenaemia, ovarian insufficiency or infertility appears in the peri- and postpubertal phase, in adolescence or in adult life [18, 20, 35, 36]. Cortisol synthesis during stress as well as aldosterone synthesis and sodium balance are not restricted significantly in those patients [18]. Finally, heterozygous carrier patients with unaffected CYP21 enzyme activity are asymptomatic.



**Figure 1:** Nomenclature and algorithm of classification of female androgenization. Diagnostic level I is the screening step regarding clarification of functional androgenization (FA), and/or of cycle irregularities, and/or of infertility, particularly in the combination with obesity (especially in adolescence). If e.g. elevated C<sup>18</sup>-sex steroid levels or low SHBG levels are found, diagnostic level II (see Material and Methods) will follow. At least on this step, areas A to C can be differentiated. In most cases, area A, the FA, will come in consideration. This area can be further divided through step II into groups FCA (skin) and FAS I (ovary), II (adrenal gland), III (multi-organ FA disorders), and IV (residual FA dysfunctions/disorders) (see Material and Methods). When very high serum levels of testosterone and/or DEHAS are found, the dexamethasone test (level III) may be used in order to differentiate a functional adrenal hyperandrogenaemia (FAS II) from a non-functional androgen excess which might suspicious for a tumorous androgenization (Area B) (data not shown). In addition, it has to be clarified on level II (e.g. by pathologic 17-hydroxy progesterone levels), whether a *CYP21A* analysis should follow (level III). On the other hand, by an accurate enquiry concerning the use of medicine just at the beginning of the medical care it will be found out whether area C (pharmaceutical androgenization) has to be considered.

PFOs: polyfollicular ovaries; DHEAS: dehydroepiandrosterone-sulphate; SHBG: sex hormone-binding globulin; A: functional androgenization; B: tumorous androgenization; C: pharmacological androgenization; FCA: functional cutaneous androgenization; FAS: functional androgenizing syndrome.

a novel classification (synonymous: grouping) based on long-term consecutive investigations.<sup>2</sup>

Therefore, the term “female androgenization” has been established as the central one with three major areas: “functional” (A), “tumorous” (B), and “pharmacological” (C) androgenization. Similar ideas of subdivision have been previously presented by Greenblatt and Augusta [50], Moltz [51], and Barbieri et al [52]. The numerically most prominent area A, functional androgenization (FA),

was further divided into 5 groups termed as: “functional cutaneous androgenization (FCA)” and “functional androgenizing syndrome (FAS) I to IV” (Fig. 1).

Patients presenting in our institution with some or all of the following symptoms, such as: cutaneous androgenetic symptoms (acne vulgaris, hirsutism and/or, androgenetic alopecia), disturbances of the menstrual cycle (particularly oligo-, amenorrhea), infertility, hyperandrogenaemia, polyfollicular ovaries (PFO), overweight/obesity and/or hyper-

insulinaemia/hyperglucosaemia were extensively investigated and analyzed retrospectively using the above mentioned systematic. The data are presented here for the first time.

## Material and Methods

### Subjects

Data of 249 premenopausal women (mean age 26.5 ± 11.3 yrs) who have been examined at the CERF were consecutively selected for the study over a 6-year period and evaluated retrospectively. The symptoms which have been the reason for patients’ clinical exploration were: acne vulgaris, hirsutism, and/or androgenetic alopecia, irregularities of the menstrual cycle (oligo-, amenorrhea), infertility, hyperandrogenaemia, decreased circulating sex hormone-binding globulin (SHBG) levels, overweight/obesity and/or hyperinsulinaemia/hyperglucosaemia and PFOs (see below).

All the subjects studied were in their early or peak reproductive phase [Staging Reproductive Aging Workshop (STRAW) stages –5 to –4] [53] apart from one woman who was found to be in the late reproductive phase (STRAW stage: –3). All individuals had not taken any hormonal or metabolic medications during the preceding 3 months aside from the treatment with iodine and or thyroxine (except for group FCA [see below] from which patients taking these medicaments were excluded). It has to be emphasized, that the diagnostic stratification presented here could be theoretically expanded over the entire range of STRAW stages –5 to +2.

### Modified Nomenclature and Novel Algorithm of Classification, Further Characterization of Patients

#### General Remarks

Female “androgenization” was used as the generic term which was further divided in 3 major areas, the areas A, B, and C, respectively (Fig. 1). Area A (FA), that summarized the numerically most

<sup>2</sup> Already in 1983, the follicular feature of the ovarian “polycystic” elements visualized by ultrasound in hyperandrogenaemic patients has been addressed [38]. Later on, grouping studies and other investigations have been performed in hyperandrogenaemic patients, and hyperandrogenaemia, hyperinsulinaemia and/or obesity have been coined to be the target points instead of using the undifferentiated common term “PCOS/D” [39, 40, 41]. A similar approach was chosen e.g. by Ehrmann et al. (1992) [42] or Azziz et al. (1998) [43] in their studies differentiating between “ovarian hyperandrogenism” and “adrenal androgen excess in hyperandrogenic women”. Later on, the term “PCOS/D” was then replaced by “functional ovarian hyperandrogenism” [44], and a classification into 4 subcategories has been introduced [7, 45, 46, 47]. In 2005, a further, slight change of the terminology with an expansion from 4 up to 5 groups followed [48], and recently a novel algorithm has been presented both in a short version [25], and in a complete one [49].

prominent kind of androgenization, will achieve exclusive attention in the present paper. A diagnostic 3-step approach was conducted. Diagnostic step I equivalent to screening was applied to patients showing mild or moderate cutaneous androgenetic symptoms, cycle irregularities, weight gain or infertility. Diagnostic step II was performed when a more pronounced cutaneous androgenization, a virilization (also called “masculinization”: severe cutaneous androgenetic symptoms, deepening of the voice, muscle hypertrophy, breast atrophy, and/or clitoromegaly), cycle irregularities (particularly oligo-, amenorrhea), PFOs, obesity, a specific family history (obesity, type 2 diabetes, “PCOS/D”, “NC-CAH” and others) and/or infertility were present; and/or when marked biochemical alterations have been found on screening step I; and/or when several of these variables have been present at the same time. Diagnostic step II is described in detail in this paper.

Based on clinical observation and the application of the current diagnostic approach [7, 25, 38, 39, 41, 44–49, 54, 55] the patients were classified in 5 groups according to the organ or organs being predominantly involved in the androgenetic symptomatology (Fig. 1).

The term “FCA” has been chosen for the first group at which the skin was found to be the major site of pathology, while no or no considerable systemic endocrine and/or metabolic factors were identifiable (peripheral androgenization).<sup>3</sup> The term “FAS” was used for the further groups.<sup>4</sup>

A set of variables were utilized including biochemical parameters (e.g. serum testosterone), symptoms (e.g. oligo-, amenorrhea) and sub-diagnoses (e.g. infertility) which consist of differently weighted anthropometric, dermatologic, endocrine,

metabolic and sonographic findings. The definitions of all variables used were listed in Table 2 apart on the description of PFO which is presented under Material and Methods, Transvaginal ultrasonography. In addition to the primary classifying variables having the potency to develop specific clusters for grouping (Tab. 3) there are some secondary (facultative) variables which were utilized for further individual characterization of each patient (Tab. 2).

A further subclassification of groups FCA and FAS I–III was regarded to be useful with subset “a” showing a full-blown feature whereas subset “b” reflected merely a non-classic minimum standard core that lacks some of the features of the complete cluster [61], and/or revealed a miscellaneous constellation (compare: [8–10, 31]). In the following, all groups will be described in detail; pathologic cut-off values of all variables (Tab. 2) were assessed by considering data of both the control ( $> \text{means} \pm \text{SD}$ ; see: Materials and Methods, Control) and of those referred references which have described same or very similar values, or considering established standards.

#### Functional Cutaneous Androgenization (FCA) (Peripheral Androgenization)

Group FCA (Fig. 1) was characterized by the presence of one, two or all three sub-variables of cutaneous androgenization: acne vulgaris, hirsutism and/or androgenetic alopecia (Tab. 2, 3). The total of cutaneous androgenization (at least one sub-variable was present) was evaluated binary and independently on the severity in this study. Detailed data of the sub-variables are not shown here. The symptom “seborrhea” which is hard to objectify as well as a special form of postmenopausal female pattern hair loss, the frontal fibrosing alopecia [68], were not considered.

In FCAa, all the other values were in the normal range except of the body mass index (BMI) the value of which was not relevant for this group. Consequently, patients of STRAW stages –2 to +2 [53] (menopausal transition – postmenopause) could be classified into group FCAa, as well. Several, not really clarified factors [71, 72] might be of relevance on hair loss with aging.

Patients were grouped under FCAb, if cutaneous androgenetic symptoms were associated with tonically elevated LH and/or increased LH/FSH ratio levels, and/or with the presence of PFOs.

#### Functional Androgenizing Syndrome (FAS) I (Ovary)

The classic constellation (see reviews: [8, 73]) that includes elevated levels of LH, of LH/FSH [39, 49, 74–79], and of T [5, 39, 49, 80, 81] with the visualization of PFOs ( $\approx$  “PCOs”) stood for group FAS Ia (Fig. 1; Tab. 3). Other parameters such as BMI were in the normal range, apart of SHBG and/or free androgen index [FAI:  $(\text{T nmol/L}/\text{SHBG nmol/L}) \times 100$ ] [82] levels which were variable. Upper cut-off levels of LH and of LH/FSH ratio are listed in Table 2; the two variables were used, since an increase of LH in combination with an enhanced FSH/LH ratio is a typical finding in STRAW stages –2 to +2 (menopausal transition to postmenopause), and, on the other hand, a decrease of LH despite an enhanced LH/FSH ratio might be found in some patients showing a trend to a hypogonadotropic state; these two endocrine constellations have nothing to do with the pathogenity of group FAS I (compare: [86]). Cut-off levels of T, of SHBG and of FAI are listed in Table 2.

Less strictly defined was the FAS Ib cluster (Fig. 1; Tab. 3) at which the typical tonic increase of LH, and/or of the LH/FSH ratio (compare: Material and Methods; Novel nomenclature and algo-

<sup>3</sup> The underlying pathology of this group was denoted in the sense of a dysfunction because pathological findings appeared to be predominantly limited on skin level without a marked systemic impact [6, 7, 56–60].

<sup>4</sup> In contrast to the term “FCA”, the substantive “syndrome” was implemented in the term “FAS” because the pathology of the “FAS” groups consists of complex dysfunctions and/or disorders on which more than one organ participates; or in other words: a syndrome was considered when “a collection of signs and features” [31] was required. A certain difficulty in the choice of an adequate nomenclature is related to the authors’ effort to implement terms whose abbreviations sound equally both in the English and German language. Other problems exist in different meanings of abbreviations: e.g. instead of FCA would the shorter term “cutaneous androgenization” result in the abbreviation “CA” which would of course be associated easily with “carcinoma”. The English term “androgen excess” is not directly suitable in the German language because the German substantive “Exzess” means something like “extremes”. “Disorder” might be applicable for both “dysfunction” and “syndrome”, and therefore the term “functional androgenetic disorder” (FAD) would make sense for all groups including “FCA” and “FAS I” to “IV”, however, the abbreviation “FAD” has got a strange sound both in English and German. The FAS groups were denoted consecutively by Roman numerals according to the predominant organs involved: ovary – FAS I; adrenal gland – FAS II; or a complex network of several implied organs (e.g. ovary, pituitary, fat tissue, pancreas, liver) – FAS III; residual dysfunctions/disorders with FA – FAS IV.

**Table 2:** Definitions of variables used in the study. Note: polyfollicular ovaries (PFOs) see: Material and Methods, Transvaginal Ultrasonography

| Primary (classifying) variables            | Definitions                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|--------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Reproductive phase                         | Staging Reproductive Aging Workshop (STRAW) stages [53]                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Menopausal transition – postmenopause      | Elevated FSH levels > 10 mIU/mL in the early follicular phase [53, 69], or > 20 mIU/mL as single annual value [70] (ovarian insufficiency WHO III) [27]                                                                                                                                                                                                                                                                                                                 |
| Acne vulgaris                              | Differentiation among open and closed comedones, papules, pustules, cysts as well as nodules [62]; a modified grading system [63] has been used: mild (I°): < 10 comedones/papules on one side of face, on forehead or nose; moderate (II°) > 10 comedones/papules and pustules on one side of face or spread to chest and/or shoulders and/or neck and/or back; severe (III°): findings described above plus deep infiltrates and/or cysts and/or nodules              |
| Hirsutism                                  | Ferriman-Gallwey-score ( $\geq 3$ –36) [30, 64, 65]                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Androgenetic alopecia [66, 67]             | Definition by the number of hairs lost per day ( $\geq 100$ /d) and by the allocation of accentuation at the scalp area: diffuse over the entire scalp (Sinclair scale 4–5) [62] or occipital, frontal, fronto-occipital (Sinclair scale 3–4) [62], as well as bitemporal or frontotemporal                                                                                                                                                                             |
| Luteinizing hormone (LH)                   | Upper cut-off serum level: 8.4 mIU/mL [83]                                                                                                                                                                                                                                                                                                                                                                                                                              |
| LH/follicle stimulating hormone ratio      | Upper cut-off level: 1.3 [39, 84, 85]                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Testosterone (T)                           | Upper cut-off serum level: 2.1 nmol/L [5, 39]; a T-value of $\geq 5.2$ nmol/L was suspicious of a severe adrenal enzyme deficiency (see below) or a C <sup>19</sup> -sex steroid secreting tumor [5, 51, 86], whereupon area B of androgenization was evident then (Fig. 1) (area B is not explained in detail in this paper)                                                                                                                                           |
| Sex hormone-binding globuline (SHBG)       | Lower cut-off serum level: 25 nmol/L [41, 87]                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Free androgen index (FAI)                  | Upper cut-off level: 5 [(T nmol/L/SHBG nmol/L) $\times$ 100] [82, 85]                                                                                                                                                                                                                                                                                                                                                                                                   |
| 17-OH-progesterone (17-OH-P)               | Baseline (0) 17-OH-P levels of $\geq 31$ nmol/L = congenital adrenal hyperplasia (CAH); ACTH stimulated 17-OH-P (17-OH-P 1) (see: Materials and Methods; Endocrine and metabolic variables) levels $\geq 31$ nmol/L: suspicious of non-classical CAH (NC-CAH) [19]; if the 17-OH-P $\Delta$ value (17-OH-P 1 minus 17-OH-P 0 in the ACTH test) is $\geq 8.1$ nmol/L [19] or the 17-OH-P 0 level is $\geq 12.1$ nmol/L [87], a mild heterozygous status might be present |
| Dehydroepiandrosterone-sulphate (DHEAS)    | Upper cut-off serum level of 9.6 $\mu$ mol/L [41] related to patients at the age < 45 yrs [93, 94]; DHEAS values of $\geq 19$ $\mu$ mol/L were suspicious of a C <sup>19</sup> -sex steroid secreting tumor [5, 51]; in such individuals, area B of androgenization is evident then (Fig. 1) (area B is not explained in detail in this paper)                                                                                                                          |
| Body mass index (BMI); overweight, obesity | Upper cut-off level: $\geq 25$ mg/kg <sup>2</sup> : overweight [103, 104]; $\geq 30$ mg/kg <sup>2</sup> : obese [103]                                                                                                                                                                                                                                                                                                                                                   |
| Glucose (fasting, post oral glucose load)  | Upper cut-off fasting serum level: 5.5 mmol/L [105, 106]; Upper cut-off 1-h post-load serum level: 7.7 mmol/L [106]                                                                                                                                                                                                                                                                                                                                                     |
| Insulin (fasting, post oral glucose load)  | Upper cut-off fasting serum level: 107 pmol/L [41, 106–108]; Upper cut-off 1-h post-load serum level: 723 pmol/L [39, 106, 109]                                                                                                                                                                                                                                                                                                                                         |
| Secondary (facultative) variables          | Definitions                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Oligomenorrhea, amenorrhea                 | Bleeding interval $\geq 36$ days, $\geq 6$ months [8]                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Infertility                                | Undesired childlessness >12 months                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Thyroid stimulating hormone                | Normal range 0.3–4 mIU/L [19]                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Cortisol                                   | Upper cut-off serum level: 690 nmol/L at 8–9 AM [19]                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Triglycerids                               | Upper cut-off serum level: 150 mg/dL [12, 13, 118, 119]                                                                                                                                                                                                                                                                                                                                                                                                                 |
| High density lipoprotein cholesterol       | Lower cut-off serum level: 50 mg/dL [12, 13, 118, 119]                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Additional variables                       | Definitions                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Prolactin                                  | Upper cut-off serum level: 425 $\mu$ U/mL [120]                                                                                                                                                                                                                                                                                                                                                                                                                         |

rhythm of classification, characterization and diagnosis; FCA) were missing [12, 13, 73, 88]. A further FAS Ib subtype was observed when 17-hydroxyprogesterone (17-OH-P)  $\Delta$ , and/or dehydroepiandrosterone-sulphate (DHEAS) levels were increased, respectively (see: Material and Methods; Novel nomenclature and algorithm of classification, characterization and diagnosis; FAS II). Thus, patients revealing a typical FAS I pattern together with e.g. functionally elevated DHEAS levels represented apparently a miscellaneous ovarian-adrenal constellation [1, 89], a feature, which was subdivided under FAS Ib because the consecutive therapy-oriented targets might

be directed predominantly through the ovarian part of endocrine insufficiency.

#### Functional Androgenizing Syndrome (FAS) II (Adrenal Gland)

The complex group FAS II consisted of two different constellations (Fig. 1; Tab. 3). The first one was caused by differently significant mutations/deletions [18, 32, 36, 49, 90]. Apart from increased T- and/or DHEAS levels, elevated 17-OH-P levels were the indicative endocrine feature; cut-off levels of baseline 17-OH-P (17-OH-P 0), of ACTH-stimulated 17-OH-P (17-OH-P 1) and of 17-OH-P  $\Delta$  value (17-OH-P 1 minus 17-OH-P 0 in the ACTH test) are

listed in Table 2. However, it has to be considered that a slight increase of 17-OH-P levels, even together with a heterozygous *CYP21A2* status, might be asymptomatic in terms of FA [90]. The same is true in cases of heterozygous carrier status with normal 17-OH-P  $\Delta$  levels. Group FAS II covered those patients who showed symptoms first during puberty or later in life. When an index case of CAH or of NC-CAH was present in the family or 17-OH-P levels had exceeded the normal range, and/or severe cutaneous androgenetic symptoms with virilization and/or strongly elevated T- or DHEAS levels (see above) were found, a *CYP21A* mutation/dele-



**Table 3:** Classification of female functional androgenization using clusters of primary variables. Note: modified from [25, 49]

| Primary variable                                      | Cluster      |   |       |                    |                    |                      |                    |                    |
|-------------------------------------------------------|--------------|---|-------|--------------------|--------------------|----------------------|--------------------|--------------------|
|                                                       | FCA          |   | FAS   |                    |                    |                      |                    |                    |
|                                                       | a            | b | I     |                    | II                 |                      | III                |                    |
|                                                       | a            | b | a     | b                  | a                  | b                    | a                  | b                  |
| Acne vulgaris, hirsutism and/or androgenetic alopecia | +            | + | –     | –                  | –                  | –                    | –                  | –                  |
| Body mass index (kg/m <sup>2</sup> )                  | –            | – | N     | N                  | –                  | –                    | ≥ 25               | ≥ 25 <sup>k</sup>  |
| PFO                                                   | ∅            | – | +     | +                  | ∅                  | –                    | +                  | +                  |
| LH (mIU/mL)                                           | N            | – | ≥ 8.5 | –                  | N                  | –                    | ≥ 8.5              | –                  |
| LH/FSH                                                | N            | – | ≥ 1.4 | –                  | N                  | –                    | ≥ 1.4              | –                  |
| T (nmol/L)                                            | N            | N | ≥ 2.2 | ≥ 2.2              | ≥ 2.2 <sup>b</sup> | N/≥ 2.2 <sup>i</sup> | ≥ 2.2              | ≥ 2.2 <sup>i</sup> |
| SHBG (nmol/L)                                         | N            | N | –     | –                  | –                  | –                    | ≤ 24               | –                  |
| FAI (T/SHBG × 100)                                    | N            | N | –     | –                  | –                  | –                    | ≥ 5.1              | ≥ 5.1 <sup>l</sup> |
| 17-OH-P (nmol/L)                                      | 0            | N | N     | N                  | ≥ 31 <sup>c</sup>  | ≥ 31 <sup>c</sup>    | N                  | N                  |
|                                                       | 1            | N | N     | N                  | ≥ 31 <sup>d</sup>  | ≥ 31 <sup>d</sup>    | N                  | N                  |
|                                                       | Δ            | N | N     | N                  | ≥ 8.1 <sup>e</sup> | ≥ 8.1 <sup>e</sup>   | N                  | –                  |
| CYP21A2 <sup>n</sup>                                  | homozygous   | ∅ | ∅     | ∅                  | + <sup>f</sup>     | + <sup>f</sup>       | ∅                  | ∅                  |
|                                                       | heterozygous | – | –     | –                  | + <sup>g</sup>     | + <sup>g</sup>       | –                  | –                  |
| DHEAS (μmol/L)                                        | N            | N | N     | ≥ 9.7 <sup>a</sup> | ≥ 9.7 <sup>h</sup> | ≥ 9.7 <sup>h</sup>   | N                  | ≥ 9.7 <sup>m</sup> |
| Glucose (mmol/L)                                      | 0            | N | N     | N                  | N                  | –                    | ≥ 5.6 <sup>i</sup> | ≥ 5.6 <sup>k</sup> |
|                                                       | 1            | N | N     | N                  | N                  | –                    | ≥ 7.8 <sup>i</sup> | ≥ 7.8 <sup>k</sup> |
| Insulin (pmol/L)                                      | 0            | N | N     | N                  | N                  | –                    | ≥ 108 <sup>j</sup> | ≥ 108 <sup>k</sup> |
|                                                       | 1            | N | N     | N                  | N                  | –                    | ≥ 724 <sup>j</sup> | ≥ 724 <sup>k</sup> |

PFO: polyfollicular ovary: maximum (max.) ovarian diameter: ≥ 31 mm, antral follicle count (follicles of 4–10 mm in max. diameter)/max. ovarian area: ≥ 8; LH: luteinizing hormone; FSH: follicle stimulating hormone; T: testosterone; SHBG: sex hormone-binding globulin; FAI: free androgen index; 17-OH-P 0: baseline 17-hydroxyprogesterone; 17-OH-P 1: 17-OH-P 60 min after adrenocorticotropin administration (ACTH test); D: 17-OH-P 1–0; glucose 0/insulin 0: baseline glucose/insulin; glucose 1/insulin 1: glucose/insulin 60 min after oral glucose loading test; FCA: functional cutaneous androgenization; FAS: functional androgenizing syndrome; a: subgroup “a” is defined by a classic full-blown cluster; b: subgroup “b” is defined by a minimum core cluster and/or a miscellaneous constellation (see below); +: presence of variable is obligate for classification; ∅: this predefined variable should not be present; –: presence of variable is not obligate for classification; N: normally ranged value of variable is obligate for classification. <sup>a</sup> Elevated DHEAS serum level in combination with a FAS Ia/b parameter cluster is subordinated in FAS Ib; this constellation reflects a miscellaneous ovarian-adrenal hyperandrogenaemic status thereby elevated DHEAS levels are of minor relevance vs. the FAS I cluster; note the age related decline in DHEAS levels [93, 94]; <sup>b–h</sup> either “b” “c–f” “d, e, g” “e, g” or “h” are required for grouping in FAS II (see also under “n”); endocrine constellation “c–f” which is typical for the postnatally found congenital adrenal hyperplasia (CAH) is usually detected postnatally; <sup>i</sup> PFO + is accepted in cases of “c–f”; such a constellation FAS IIb reflects a miscellaneous adrenal-ovarian constellation, the presence of PFO being of minor importance vs. the FAS IIb “c–f” cluster; <sup>j</sup> at least one variable of index “j” is required for grouping in FAS IIIa; <sup>k</sup> at least one variable of index “k” as well as at least one variable of index “l” are required for grouping in FAS IIIb; <sup>m</sup> an elevated DHEAS serum level in combination with a FAS IIIa/b variable cluster is subordinated in FAS IIIb; this constellation reflects a miscellaneous ovarian-adrenal hyperandrogenaemic status with anthropometric and/or metabolic dysfunctions thereby the elevation of the DHEAS level is of minor relevance vs. the FAS III cluster; <sup>n</sup> the determination of CYP21A2 is carried out as diagnostic step III (s. Material and Methods); if a CYP21A2-mutation/deletion is not found, increased values of 17-OH-P 1 and 17-OH-P Δ are without any clinical relevance anymore.

tion analysis was performed (diagnostic step III, detailed data are not shown here), particularly in infertile patients. Very rare cases of CYP11 or CYP17 deficiencies are not investigated here but need the referred attention if necessary.

The second constellation revealed elevated DHEAS levels [43] that might be caused by mild 3β-hydroxysteroid dehydrogenase deficiency [91] or an exaggerated adrenocortical Δ<sup>5</sup>-17-hydroxylase

activity [92]; 17-OH-P levels were in the normal range. The cut-off DHEAS level is listed in Tables 2 and 3. Regarding the two constellations of FAS IIa, levels of LH and LH/FSH ratio were in the normal range, and PFOs were not present.

A FAS IIb adrenal-ovarian sub-phenotype was introduced in cases of significant CYP21A2 mutations/deletions with the concomitant presence of a PFO; a clinically adrenal dominance of FA was

presumed in these cases in contrast to the FAS Ib ovarian-adrenal subtype (see above) where the ovarian part was considered to be predominant. Furthermore, the classifying criteria were less strict in group FAS IIb, since LH, LH/FSH ratio, glucose and/or insulin levels (see below) were variable; the latter constellations comprised patients with a mix of adrenal FA and metabolic disturbances; a direct association e.g. between circulating DHEAS and insulin is questionable [95], and might reflect rather a random constellation.

#### Functional Androgenizing Syndrome (FAS) III (Multi-Organ-Disorders with FA, Obesity, Hyperinsulinaemia)

The FAS IIIa cluster was comparable to that subset of „PCOS/D“ which is associated with MetS as proposed by the RCO3 [12, 13] (Fig. 1; Tab. 3). The realization of this classic phenotype which has been described first by Stein and Leventhal [96] and in numerous publications respecting different aspects later on [39, 83, 97–102] has actually triggered the awareness on the entire syndrome.

In contrast to group FAS Ia, FAS IIIa patients were overweight or obese (Tab. 2). Dysfunction of the glucose and/or insulin metabolism were present (Tab. 2) defined by an increase of glucose and/or insulin levels; it has to point out that the term “hyperinsulinaemia” is not synonymous to the term “insulin resistance” which is therefore not used here. There is a reverse interrelationship among SHBG, a primary (classifying) variable of the FAS IIIa cluster, and both insulin [98, 107, 110–115] and body weight [116].

Minimum standard core phenotypes were assigned to group FAS IIIb (Fig. 1; Tab. 3): LH, LH/FSH and SHBG levels were variable; either an increased BMI or alterations of circulating glucose and/or insulin levels were found. Similarly to the miscellaneous ovarian-adrenal FAS Ib phenotype (see above), FAS III pattern patients with elevated DHEAS levels were grouped in FAS IIIb.

#### Functional Androgenizing Syndrome (FAS) IV (Residual Dysfunctions/Disorders with FA)

Due to the enormous heterogeneity of FA a clear assignment into a pathogenic overall concept was not possible in each patient; furthermore, it did not make



**Table 4:** Differential diagnosis of female androgenization. Note: modified from [49]; no demand for completeness.

| Variable, dysfunction, disease, syndrome | Variables belonging to female functional androgenization |      |           |          |                     |                     |                                  |
|------------------------------------------|----------------------------------------------------------|------|-----------|----------|---------------------|---------------------|----------------------------------|
|                                          | PFO                                                      | Acne | Hirsutism | Alopecia | Hyper-androgenaemia | Obesity, overweight | Hyperinsulinaemia, IGT, diabetes |
| Pubertal acne                            |                                                          | +    |           |          |                     |                     |                                  |
| Acne inversa                             |                                                          | +    |           |          |                     |                     |                                  |
| Sjögren-Larsson syndrome                 |                                                          | +    |           |          |                     |                     |                                  |
| Hypertrichiosis                          |                                                          |      | +         |          |                     |                     |                                  |
| Thyroid disorders                        |                                                          |      | +         | +        |                     | +                   |                                  |
| Anorexia nervosa                         |                                                          |      | +         | +        |                     |                     |                                  |
| M. Cushing                               |                                                          |      | +         | +        | +                   | +                   | +                                |
| Cushing syndrome                         |                                                          |      | +         | +        | +                   | +                   | +                                |
| CAH                                      |                                                          |      | +         |          | +                   |                     |                                  |
| Pubertas praecox                         |                                                          |      | +         | +        | +                   |                     |                                  |
| MAS                                      |                                                          |      | +         |          |                     |                     |                                  |
| PAIS                                     |                                                          |      | +         |          | +                   |                     |                                  |
| Effluvium (e.g. postpartal)              |                                                          |      |           | +        |                     |                     |                                  |
| Alopecia areata                          |                                                          |      |           | +        |                     |                     |                                  |
| Alopecia totalis                         |                                                          |      |           | +        |                     |                     |                                  |
| Adrenal gland insufficiency              |                                                          |      |           | +        |                     |                     |                                  |
| Multicystic ovaries                      | +                                                        |      |           |          |                     |                     |                                  |
| PAS I/II                                 |                                                          |      |           | +        |                     |                     | +                                |
| CSTS                                     |                                                          |      |           | +        |                     |                     |                                  |
| Acromegaly                               |                                                          |      | +         |          |                     |                     |                                  |
| Healthy obesity, Overweight              |                                                          |      |           |          |                     | +                   |                                  |
| Obesity syndromes:                       |                                                          |      |           |          |                     | +                   |                                  |
| – PWS                                    |                                                          |      |           |          |                     | +                   |                                  |
| – Ahlstrom syndrome                      |                                                          |      | +         |          |                     | +                   |                                  |
| – Cohen syndrome                         |                                                          |      |           |          |                     | +                   |                                  |
| – Carpenter syndrome                     |                                                          |      |           |          |                     | +                   |                                  |
| T1DM                                     |                                                          |      |           |          |                     |                     | +                                |
| T2DM                                     |                                                          |      |           |          |                     |                     | +                                |
| MODY                                     |                                                          |      |           |          |                     |                     | +                                |
| Leprechaunism                            |                                                          |      |           |          |                     |                     | +                                |
| PC-1-Mutation                            |                                                          |      |           |          |                     | +                   |                                  |

PFO: polyfollicular ovaries; IGT: impaired glucose tolerance; CAH: congenital adrenal hyperplasia; MAS: McCune-Albright syndrome; PAIS: partial androgen insensitivity syndrome; PAS: polyglandular autoimmune syndrome; CSTS: ectodermal (hypo-anhidrotic) dysplasia (Christ-Siemens-Touraine-syndrome); PWS: Prader-Willi syndrome; T1DM: type 1 diabetes mellitus; T2DM: type 2 DM; MODY: mature onset diabetes of the young; PC-1: prohormone convertase 1.

sense to set up additional subsets with a very small number of special cases. Individuals with such variable profiles belonged into group FAS IV (Fig. 1), e.g. obese FA-patients with increased FAI without the visualization of PFOs.

### Further Characterization

Patients' individual characterization was additionally considered by the introduction of secondary (facultative) variables (Tab. 2). These factors did not accord group-determining function because of their missing discriminating specificity, or some of them were not directly involved in FA. Patients' final diagnosis comprised both classification and individual characterization [25, 49, 117]. This is exemplified with the symptom oligomenorrhea or amenorrhea (Tab. 2). Since cycle dysfunctions occur with all kinds of ovarian insufficiencies [27, 48] this variable is unspecific but may be nevertheless of diagnostic relevance in

FA-patients (data not shown). Infertility (Tab. 2) (data not shown) represents a fundamental sub-diagnosis, however, it does not serve as a FA-specific primary variable. Lipids do not have a direct impact on FA, however, their determination supports the complete diagnosis of MetS in FAS III; cut-off levels of triglycerides and of high density lipoprotein cholesterol (HDL) as well as of cortisol, further essential endocrine variables, are listed in Table 2. Detailed data out of the set of secondary variables are presented concerning lipids (see below). Serum prolactin level was measured additionally for completion the endocrine check-up (additional variables Tab. 2).

### Differential Diagnosis for Exclusion

Differential diagnosis for exclusion is listed in Table 4. Their FA-associated symptoms are of minor relevance among the overall symptomatology, e.g. hirsut-

ism and obesity in cases of hyperthyreosis or M. Cushing [19]; or androgenetic symptoms are independent on any well defined endocrine or metabolic dysregulation, e.g. hair loss in the case of alopecia areata [121], or acneiform ichthyosis in the case of Sjögren-Larsson syndrome [122]; or primary variables are prominent factors of non-FA-disorders, e.g. obesity in cases of monogenetic obesity syndromes such as Prader-Willi syndrome [123].

In summary, we tested a new classification based on biochemical and clinical classifiers in a large number of patients with FA

### Control

A control group consisting of 14 premenopausal, healthy women without any signs of FA was formed. The volunteers should have had a history of  $\geq 3$  regular menstrual cycles ( $\geq 21$  to  $\leq 35$

days); mean circulating progesterone values obtained around day 7 prior the expected approaching menstrual bleeding were  $\geq 31$  nmol/L through which a sufficient luteal phase was proven. Major blood sampling (see: Materials and Methods, Endocrine and metabolic parameters) was performed between days 3 to 7 of the following second menstrual cycle (earlyfollicular phase). The early- to midfollicular phase was hormonally confirmed by serum levels of estradiol  $\leq 440$  pmol/L and of progesterone  $\leq 6$  nmol/L, respectively [124]. In 8 out of the 14 volunteers an extended investigation was performed (see: Materials and Methods; Endocrine parameters). None of the volunteers had taken hormonal or metabolic medications during the preceding 3 months. Each volunteer gave a written consent for the study.

### Transvaginal Ultrasonography

Ultrasonography of the ovaries using a vaginal ultrasound probe (Sonoline SI 200, Siemens; Germany) was performed at the same time of blood sampling (see: Materials and Methods; Endocrine and metabolic parameters). Based on current literature regarding ultrasonographic findings of the ovaries [8, 73, 125] and on own studies [55], a PFO, a term which corresponds to the so called "PCO", was defined if the maximum (max.) ovarian diameter was  $\geq 28$  mm, and the antral follicle count (follicles of 4 to 10 mm in max. diameter)/max. ovarian area was  $\geq 8$ . The latter sonographic parameter corresponded to reports on an excessive number of pre-antral and antral follicles found in ovaries of the so called "PCOS" [126, 127]. A further condition of the PFO/"PCO" was a stroma/total area ratio  $> 0.33$  [55, 128]; if the latter subvariable was  $\leq 0.33$ , the max. ovarian diameter must be  $\geq 31$  mm. The presence of PFOs was evaluated binary, thereby the visualization of at least one side (unilateral)/individual was considered positive [129]. It has to be recognized that an PFO/"PCO" is according to Netter (1990) [21] just a "clinical finding similar to splenomegaly or hepatomegaly; cystic ovaries are found in a multiplicity of states, which have nothing in common etiologically; the clinical management varies and is dependent on the etiology". The severe, diffuse form of luteinized and hyperplastic ovarian stroma, the ovarian hyperthecosis [130], has been not clearly defined sonogra-

phically in literature so far. In the present study, hyperthecosis was diagnosed when the max. diameter of an ovary that reveals a homogeneous internal structure, was  $\geq 35$  mm; this special form was subdivided then under the term "PFO". The finding "PFO" has to be clearly distinguished from real "multicystic" formations which is typical e.g. for ovarian cystadenoma [131].

### Endocrine and Metabolic Variables

Vein blood sampling (and also ovarian ultrasound, see: Subjects; Control) was performed between days 3 to 7 of regular menstrual cycles and at random in oligo-amenorrhea. Patients were enrolled in the study only if the definition of the early- to midfollicular phase used for the control (see under Control) was fulfilled. After an overnight (12 h) fast, vein blood samples were obtained between 8 AM and 9 AM at 20 min intervals (= baseline value 0, e.g. 17-OH-P 0). Blood samples were centrifuged, and sera of the baseline value (0) were pooled equally and either processed immediately and/or stored at  $-20^{\circ}\text{C}$  until assayed. All serum parameters were determined by commercially available immunoassay kits: intra- and inter-assay variations for all assays were  $< 6\%$  and  $< 10\%$ , respectively [39].

In addition to the determination of baseline serum levels (0), the following further endocrine test procedure was performed in all patients, and in 8 out of the 14 volunteers: i. immediately after last baseline blood sampling, an oral administration of 75 g glucose (Dextro® O.G.T.; Roche, Germany) was applied, and another blood sample was collected one hour later to determine serum glucose (Gluc 1) and insulin (Ins 1) (oral glucose loading test [oGLT]); this test was performed in order to estimate fasting and/or glucose-stimulated glucose and insulin levels [39, 106]; ii. thereafter, 250  $\mu\text{g}$  ACTH (Synacthen®; Novartis Pharma, Germany) was administered iv over 3 minutes, and another blood sample was collected one hour later to determine serum 17-OH-P  $\Delta$  (ACTH test) as an endocrine marker of *CYP21A2* deficiency [43, 19]. Each cut-off level was presented above already (Tab. 2, 3).

### Statistics

The data analysis was performed using SAS V 8 for Windows. Means  $\pm$  SD was

used for statistical analysis of numeric variables. Although a marked dispersion of the values of some variables was observed the median was not presented in these cases, because it would be without any changes concerning statistical evidence. Regarding the variable "age" the unpaired t-test was performed. All other numeric parameters could not be assumed as normally distributed, having performed the Wilcoxon rank-sum test. Fisher's 2-sided exact tests were performed comparing the categorical parameters.  $P < 0.05$  was considered statistically significant. To predict the parameters of interest the linear and multiple linear regression was performed. In the multiple regression analyses the backward selection procedure at the level 0.1 was used.

## Results

### General Overview of Patients vs. Control

Based on the criteria defined in Material and Methods 126 out of the 249 patients have been enrolled finally in the study. The reason for patients' exclusion were: incomplete data, elevated serum levels of estradiol ( $> 440$  pmol/L) and/or progesterone ( $> 6$  nmol/L) indicating the presence of a dominant follicle or luteinization (see Material and Methods, Control, and Endocrine and Metabolic Variables, and below) at the time of testing, missing adrogenetic variables, and various disorders enumerated under differential diagnosis (Tab. 4), respectively. Patients were slightly but significantly younger than controls (C) (Tab. 5). As part of the definition described above, early- to midfollicular phase related serum levels of estradiol and progesterone were confirmed in both the patient group and C ( $161 \pm 95$  pmol/L vs.  $184 \pm 113$  pmol/L, and  $2.8 \pm 1.4$  nmol/L vs.  $2.3 \pm 0.9$  nmol/L, both non significant [NS]). Prolactin levels were lower in patients vs. control ( $254 \pm 131$   $\mu\text{IU/mL}$ , and  $382 \pm 180$   $\mu\text{IU/mL}$ ;  $P = 0.0100$ ); in 8 out of the 126 patients, prolactin levels were elevated ranging between 457 and 809  $\mu\text{IU/mL}$ ; a significant allocation of elevated prolactin levels to one of the groups was not found.

Overall values of the primary (classifying) variables in patients vs. C were given in Table 5. Cutaneous androgenetic symptoms were present in the ma-

**Table 5:** Overall results of primary variables in patients with functional androgenization vs. control. Values are means  $\pm$  SD.

| Primary variable                  |            | Patients<br>(n = 126) | Control<br>(n = 14) | P-value   |
|-----------------------------------|------------|-----------------------|---------------------|-----------|
| Age (yrs)                         |            | 25.8 $\pm$ 5.4        | 29.8 $\pm$ 5.0      | 0.0091    |
| BMI (kg/m <sup>2</sup> )          |            | 26.7 $\pm$ 6.2        | 20.7 $\pm$ 1.8      | < 0.0001  |
| Cutaneous androgenization [n (%)] |            | 94 (74.6)             | 0 (0)               | < 0.0001  |
| PFO [n (%)]                       |            | 103 (81.8)            | 5 (35.7)            | 0.0005    |
| LH (mIU/mL)                       |            | 11.8 $\pm$ 5.6        | 5.6 $\pm$ 2.5       | < 0.0001  |
| LH/FSH                            |            | 2.3 $\pm$ 1.1         | 1.0 $\pm$ 0.4       | < 0.0001  |
| T (nmol/L)                        |            | 2.9 $\pm$ 1.1         | 1.3 $\pm$ 0.5       | < 0.0001  |
| SHBG (nmol/L)                     |            | 25 $\pm$ 18.9         | 50 $\pm$ 15         | < 0.0001  |
| FAI (T/SHBG $\times$ 100)         |            | 24.7 $\pm$ 30.0       | 2.8 $\pm$ 1.4       | < 0.0001  |
| 17-OH-P (nmol/L)                  | 0*         | 6.6 $\pm$ 16.9        | 2.7 $\pm$ 1.2       | < 0.00001 |
|                                   | 1*         | 13.6 $\pm$ 31.3       | 5.3 $\pm$ 2.7       | 0.0030    |
|                                   | $\Delta$ * | 6.96 $\pm$ 15.6       | 2.25 $\pm$ 3.1      | 0.0497    |
| CYP21A2 [n (%)]                   |            | 40 (31.8)             | 2 (14.3)            | NS        |
| DHEAS ( $\mu$ mol/L)              |            | 6.6 $\pm$ 2.8         | 3.8 $\pm$ 1.6       | 0.0001    |
| Glucose (mmol/L)                  | 0*         | 4.6 $\pm$ 0.9         | 4.7 $\pm$ 0.2       | NS        |
|                                   | 1*         | 6.5 $\pm$ 2.5         | 5.1 $\pm$ 1.5       | NS        |
| Insulin (pmol/L)                  | 0*         | 94 $\pm$ 130          | 31 $\pm$ 18         | 0.0001    |
|                                   | 1*         | 682 $\pm$ 523         | 205 $\pm$ 157       | 0.0005    |

\* Determined in 8 out of 14 volunteers; NS: not statistically significant. For abbreviations see Table 3.

**Table 6:** Correlation of body mass index and free androgen index with glucose and insulin. A multiple regression analysis.

| Variable  | P-value                     |        |
|-----------|-----------------------------|--------|
|           | BMI<br>(kg/m <sup>2</sup> ) | FAI    |
| Glucose 0 | 0.0002                      | 0.1285 |
| Glucose 1 | < 0.0001                    | 0.8278 |
| Insulin 0 | 0.0007                      | 0.0494 |
| Insulin 1 | < 0.0001                    | 0.0044 |

Glucose and insulin 0: fasting values; glucose and insulin 1: 1-h post-load values; BMI: body mass index; FAI: free androgen index.

majority of patients; mostly hirsutism, followed by acne vulgaris and androgenetic alopecia (detailed data not shown). Glucose levels and the frequency of CYP21A2 mutation/deletion were not significantly different; SHBG levels were significantly lower, while all other parameters were found to be significantly elevated in the patient group vs. C. A correlation between DHEAS and T levels was not observed ( $p = 0.2$ ). In order to verify the dependence of PFO on T and LH levels, a multiple regression analysis with backward selection procedure was performed. With a R<sup>2</sup> of 0.12, this model explains approximately only 12 % of the variability in PFO. By mul-

tipple regression analysis a highly significant correlation could be found between the presence of PFO and LH levels ( $p < 0.0001$ ) but not between PFO and testosterone levels. Multiple regression analysis also revealed a significant correlation of both BMI and FAI with fasting and 1-h post-load levels of insulin, while only BMI displayed a significant correlation with the respective glucose levels (Table 6). These models explain 15 % and 31 % of the variability of glucose 0 and glucose 1.

Regarding secondary (facultative) variables overall results are shown in Table 7; detailed data are presented for lipids only (Fig. 3).

## Classification (Grouping)

### Overview

Results following classification into the 5 groups (Fig. 1) by using the primary (classifying) variables (Tab. 3) are shown in Table 8 and Figure 2. Patients of groups FAS I, FAS II, FAS IV and FCA were significantly younger vs. C ( $p = 0.0066, 0.0127, 0.0268$  and  $0.0264$ , respectively). Cutaneous androgenetic symptoms were – by definition – significantly more frequently present in all groups vs. C (Tab. 8) (data as the sub-variables such as acne vulgaris, hirsutism and androgenetic alopecia are not shown here). The results show clearly that five statistically distinctive groups (entities) can be discerned which are presented in detail in the following sections. Because of the strong statistical power of the largest groups, FAS I and FAS III, data of their subsets “a” and “b” are presented additionally (Tab. 9).

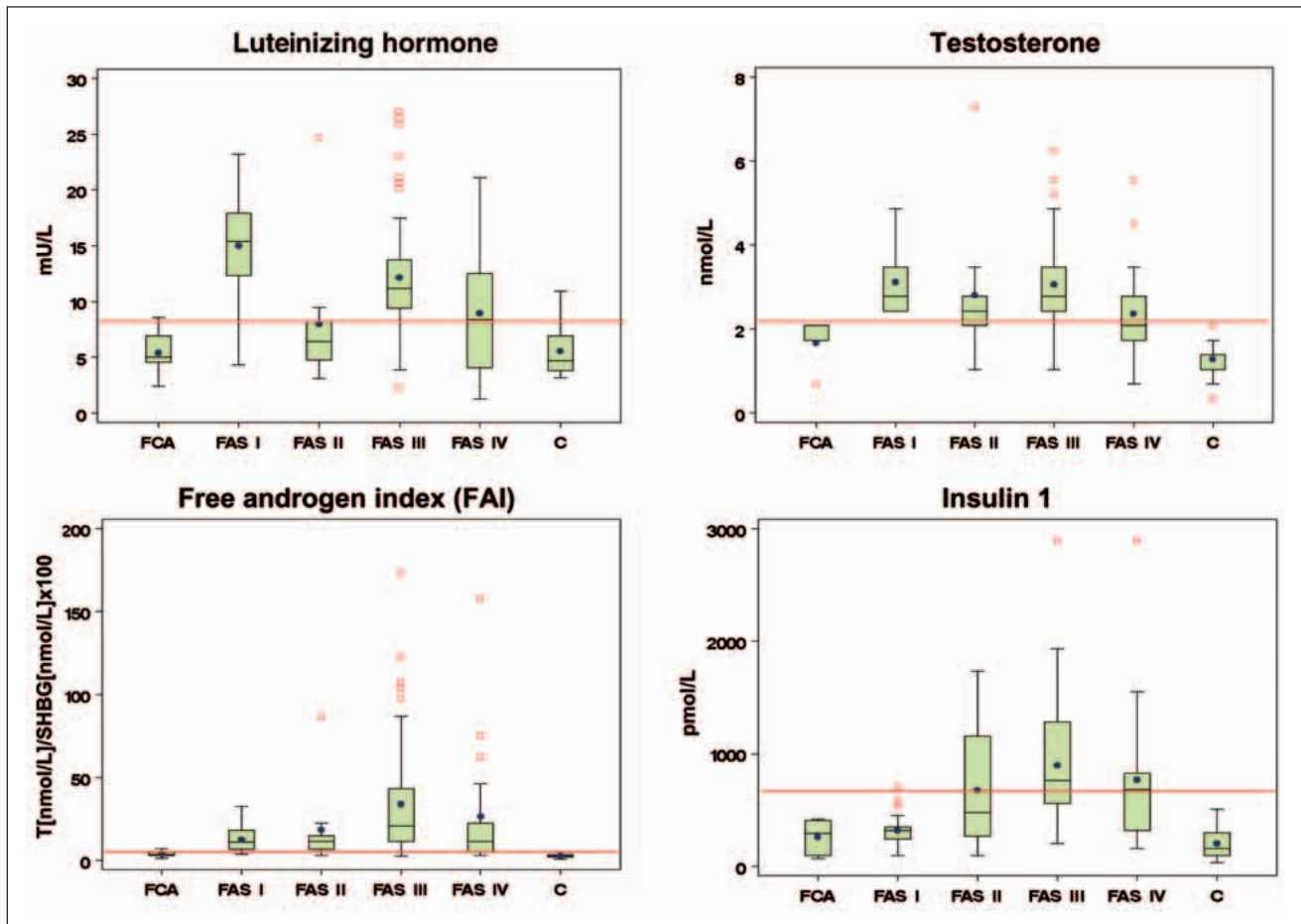
### Functional Cutaneous Androgenization (FCA) (Peripheral Androgenization)

Based on the definitions given with the clusters in Table 3, group FCA was characterized predominantly by cutaneous androgenization (100 %) (Tab. 8); its presence, however, was not significantly different among the groups. One woman out of the 6 FCA patients showed a “pure” constellation (FCA a) with normally ranging endocrine and metabolic variables and without the presence of PFOs. In the residual 5 individuals, PFOs were visualized (FAC b). Therefore, the frequency of PFOs in patients with FCA was significantly higher vs. FAS IV, and significant differences in the presence of PFOs in FCA vs. the

**Table 7:** Overall results of secondary variables in patients with functional androgenization vs. control. Values are means  $\pm$  SD.

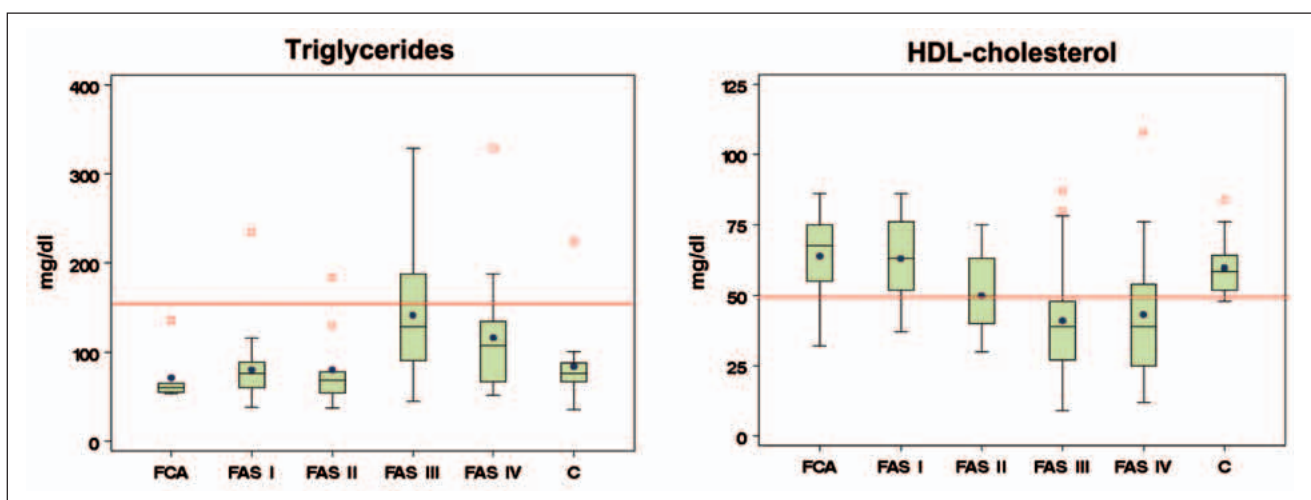
| Secondary variable                      | Patients<br>(n = 126) | Control<br>(n = 14) | P-value |
|-----------------------------------------|-----------------------|---------------------|---------|
| Age (yrs)                               | 25.8 $\pm$ 5.4        | 29.8 $\pm$ 5.0      | 0.0091  |
| Oligo-, amenorrhea [n (%)]              | 109 (86.51)           | 0 (0)               | –       |
| Follicle stimulating hormone (mIU/mL)   | 5.1 $\pm$ 1.4         | 6.0 $\pm$ 2.2       | NS      |
| Thyroid stimulating hormone (mIU/L)*    | 1.7 $\pm$ 1.6         | 1.7 $\pm$ 0.7       | NS      |
| Cortisol (nmol/L)                       | 436 $\pm$ 163         | 413 $\pm$ 116       | NS      |
| Triglycerids (mg/dL)*                   | 114 $\pm$ 61          | 84 $\pm$ 43         | NS      |
| High density lipoprotein (HDL) (mg/dL)* | 49 $\pm$ 20           | 60 $\pm$ 10         | 0.0223  |

\* Values of thyroid stimulating hormone have been determined under thyroid hormone therapy in a considerable number of patients; \* determined in 8 out of 14 volunteers; NS: not statistically significant. For abbreviations see Table 3.



**Figure 2:** Box plots of selected major primary (classifying) variables in all groups of patients with functional androgenization. Results of classification of functional androgenization summarizing full-blown and minimum standard core/miscellaneous clusters (subgroups a and b, respectively). Statistically significant differences among the groups are shown in detail in Table 8.

FCA: functional cutaneous androgenization; FAS: functional androgenizing syndrome. Insulin 1: 1-h post-load insulin. Results are shown as median (line within in the box), mean (dot within the box), 75<sup>th</sup> and 25<sup>th</sup> percentiles (upper and lower limits of the box), maximum observation below upper fence (T), minimum observation above lower fence (L) and maximum observation (□). Dotted lines represent the respective cut-off values: LH (8.4 mU/mL), testosterone (2.1 nmol/L), free androgen index (5), serum insulin 1 (723 pmol/L).



**Figure 3:** Box plots of serum lipids, the major secondary (facultative) variable, in all groups of patients with functional androgenization.

For legend see Figure 2. Dotted lines represent the respective cut-off values: triglycerides (150 mg/dL), high density lipoprotein (HDL)-cholesterol (50 mg/dL).

Statistically significant differences are:

|              |                   |                  |     |                    |                          |
|--------------|-------------------|------------------|-----|--------------------|--------------------------|
| Triglycerids | FCA vs. III, IV   | 0.0021, 0.0301   | HDL | FAS I vs. II–IV    | 0.0306, < 0.0001, 0.0003 |
|              | FAS I vs. III, IV | < 0.0001, 0.0161 |     | FAS III vs. FCA, C | 0.0173, 0.0001           |
|              | FAS III vs. II, C | 0.0014, 0.0003   |     | FAS IV vs. C       | 0.0030                   |



**Table 8:** Results of patients' classification summarizing full blown and minimum standard core/miscellaneous clusters (subgroups "a" and "b," respectively). Values are means  $\pm$  SD.

| Primary variable                  |            | FCA<br>(n = 6)  | FAS I<br>(n = 33) | FAS II<br>(n = 10) | FAS III<br>(n = 59) | FAS IV<br>(n = 18) | Control<br>(n = 14) |
|-----------------------------------|------------|-----------------|-------------------|--------------------|---------------------|--------------------|---------------------|
| Age (yrs)                         |            | 24.0 $\pm$ 4.7  | 25.5 $\pm$ 4.7    | 24.0 $\pm$ 5.4     | 26.8 $\pm$ 5.4      | 24.9 $\pm$ 6.4     | 29.8 $\pm$ 5.0      |
| BMI (kg/m <sup>2</sup> )          |            | 22.0 $\pm$ 1.6  | 21.4 $\pm$ 1.9    | 25.0 $\pm$ 5.3     | 30.0 $\pm$ 5.3      | 28.3 $\pm$ 7.4     | 20.7 $\pm$ 1.8      |
| Cutaneous androgenization [n (%)] |            | 6 (100.0)       | 23 (69.7)         | 8 (80.0)           | 43 (72.9)           | 14 (77.8)          | 0 (0)               |
| PFOs [n (%)]                      |            | 5 (83.3)        | 33 (100.0)        | 3 (30.0)           | 59 (100.0)          | 3 (16.7)           | 5 (37.5)            |
| LH (mIU/mL)                       |            | 5.4 $\pm$ 2.1   | 15 $\pm$ 4.5      | 8.0 $\pm$ 6.2      | 12.2 $\pm$ 5.2      | 9.0 $\pm$ 5.3      | 5.6 $\pm$ 2.5       |
| LH/FSH                            |            | 1.1 $\pm$ 0.4   | 2.8 $\pm$ 1.0     | 1.5 $\pm$ 1.1      | 2.5 $\pm$ 1.0       | 1.8 $\pm$ 0.9      | 1.0 $\pm$ 0.4       |
| T (nmol/L)                        |            | 1.7 $\pm$ 0.5   | 3.1 $\pm$ 0.7     | 2.8 $\pm$ 1.7      | 3.1 $\pm$ 1.1       | 2.4 $\pm$ 1.2      | 1.3 $\pm$ 0.5       |
| SHBG (nmol/L)                     |            | 48.3 $\pm$ 15.5 | 34.2 $\pm$ 19.8   | 25.3 $\pm$ 15.3    | 18.3 $\pm$ 16.1     | 22.3 $\pm$ 16.7    | 50.4 $\pm$ 14.8     |
| FAI (T/SHBG $\times$ 100)         |            | 3.9 $\pm$ 2.1   | 12.7 $\pm$ 7.7    | 18.6 $\pm$ 24.5    | 34.0 $\pm$ 34.0     | 26.6 $\pm$ 38.8    | 2.8 $\pm$ 1.4       |
| 17-OH-P (nmol/L)                  | 0*         | 3.2 $\pm$ 2.1   | 5.5 $\pm$ 2.0     | 26.1 $\pm$ 58.5    | 5.0 $\pm$ 2.3       | 4.2 $\pm$ 2.2      | 2.7 $\pm$ 1.2       |
|                                   | 1*         | 6.3 $\pm$ 2.1   | 8.8 $\pm$ 3.4     | 58.0 $\pm$ 104.4   | 11.0 $\pm$ 5.2      | 8.5 $\pm$ 4.7      | 5.3 $\pm$ 2.7       |
|                                   | $\Delta$ * | 3.1 $\pm$ 1.0   | 3.3 $\pm$ 2.6     | 31.8 $\pm$ 49.6    | 6.0 $\pm$ 4.0       | 4.3 $\pm$ 4.7      | 2.2 $\pm$ 3.1       |
| CYP21A2 [n (%)]                   |            | 1 (16.7)        | 10 (30.3)         | 6 (60.0)           | 18 (30.5)           | 5 (27.8)           | 2 (14.3)            |
| DHEAS ( $\mu$ mol/L)              |            | 4.3 $\pm$ 1.6   | 5.8 $\pm$ 2.7     | 10.2 $\pm$ 2.6     | 6.8 $\pm$ 2.4       | 5.9 $\pm$ 2.5      | 3.8 $\pm$ 1.6       |
| Glucose (mmol/L)                  | 0*         | 4.5 $\pm$ 0.6   | 4.3 $\pm$ 0.6     | 5.0 $\pm$ 1.4      | 4.6 $\pm$ 1.0       | 4.8 $\pm$ 0.9      | 4.7 $\pm$ 0.2       |
|                                   | 1*         | 5.3 $\pm$ 1.6   | 4.7 $\pm$ 1.3     | 7.4 $\pm$ 2.1      | 7.4 $\pm$ 2.6       | 7.0 $\pm$ 2.7      | 5.1 $\pm$ 1.5       |
| Insulin (pmol/L)                  | 0*         | 41 $\pm$ 25     | 45 $\pm$ 18       | 150 $\pm$ 269      | 116 $\pm$ 141       | 100 $\pm$ 95       | 31 $\pm$ 18         |
|                                   | 1*         | 264 $\pm$ 157   | 321 $\pm$ 122     | 678 $\pm$ 530      | 900 $\pm$ 514       | 771 $\pm$ 661      | 205 $\pm$ 157       |

\* Determined in 8 out of 14 volunteers. For abbreviations see Table 3.

**Statistically significant differences among the groups in Table 8.**

| Variable             | Group, control                                                                                                | P-value                                                                                      | Variable         | Group, control                                                                                   | P-value                                                                                     |
|----------------------|---------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|------------------|--------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| Cut. androgenization | FCA, FAS I-IV vs. FAS C                                                                                       | < 0.0001                                                                                     | 17-OH-P 0        | FCA vs. FAS I-III<br>FAS I vs. IV, C<br>FAS II-IV vs. C                                          | 0.0078, 0.0168, 0.0161<br>0.0065, < 0.0001<br>0.0008, < 0.0001, 0.0184                      |
| BMI                  | FCA vs. FAS III<br>FAS I vs. II-IV<br>FAS II vs. III, C<br>FAS III, IV vs. C                                  | 0.0005<br>0.0101, < 0.0001, 0.0011<br>0.0029, 0.0128<br>< 0.0001, 0.0025                     | 17-OH-P 1        | FCA vs. FAS III, IV<br>FAS I vs. II, C<br>FAS II vs. IV, C<br>FAS III vs. C                      | 0.0047, 0.0107<br>0.0139, 0.0148<br>0.0227, 0.0018<br>0.0009                                |
| PFO                  | FCA vs. FAS IV                                                                                                | 0.0069                                                                                       | 17-OH-P $\Delta$ | FCA vs. FAS II, III<br>FAS I vs. II, III<br>FAS II, III vs. C                                    | 0.0344, 0.0208<br>0.0031, 0.0005<br>0.0113, 0.0126                                          |
| LH                   | FCA vs. FAS I, III<br>FAS I vs. II-IV, C<br>FAS II vs. III<br>FAS III vs. IV, C                               | 0.0004, 0.0010<br>0.0008, 0.0010, 0.0003,<br>< 0.0001<br>0.0017<br>0.321, < 0.0001           | CYP21A2          | FAS II vs. C                                                                                     | 0.0324                                                                                      |
| LH/FSH               | FCA vs. FAS III, IV<br>FAS I vs. FCA,<br>FAS II, IV, C<br>FAS II vs. III<br>FAS III vs. IV, C<br>FAS IV vs. C | 0.0004, 0.0402<br>0.0005, 0.0020, 0.0030,<br>< 0.0001<br>0.0020<br>0.0156, < 0.0001<br>0.007 | DHEAS            | FCA vs. FAS III<br>FAS I vs. III, C<br>FAS II vs. FCA,<br>FAS I, III, IV, C<br>FAS III, IV vs. C | 0.0131<br>0.0372, 0.0095<br>0.0040, 0.0005, 0.0010,<br>0.0007, < 0.0001<br>< 0.0001, 0.0176 |
| T                    | FCA vs. FAS II<br>FAS I vs. FCA,<br>FAS II, IV, C<br>FAS III vs. FCA, FAS IV, C<br>FAS II, IV vs. C           | 0.0452<br>0.0001, 0.0500, 0.0023,<br>< 0.0001<br>0.0009, 0.0086, < 0.0001<br>0.0013, 0.0021  | Gluc 1           | FCA vs. FAS III<br>FAS I vs. II-IV<br>FAS II-IV vs. C                                            | 0.0322<br>0.0008, < 0.0001, 0.0005<br>0.0367, 0.0074, 0.0424                                |
| SHBG                 | FCA vs. FAS I-IV<br>FAS I vs. III, IV<br>FAS I-IV vs. C                                                       | 0.0450, 0.0262, 0.0008,<br>0.0059<br>< 0.0001, 0.0253<br>0.0038, 0.0020, < 0.0001,<br>0.0002 | Insulin 0        | FCA vs. FAS III<br>FAS I vs. III, IV, C<br>FAS II-IV vs. C                                       | 0.0050<br>< 0.0001, 0.0022, 0.0466<br>0.0070, < 0.0001, 0.0082                              |
| FAI                  | FCA vs. FAS I-IV<br>FAS I vs. III, C<br>FAS II-IV vs. C                                                       | 0.0012, 0.0147, 0.0002,<br>0.0069<br>0.0002, < 0.0001<br>0.0002, < 0.0001, < 0.0001          | Insulin 1        | FAS I vs. C<br>FAS II vs. I, C<br>FAS III vs. FCA, I, C<br>FAS IV vs. FCA, FAS I, C              | 0.0287<br>0.0480, 0.0235<br>0.0010, < 0.0001, < 0.0001<br>0.0402, 0.0039, 0.0027            |

**Table 9:** Results of patients' classification regarding full-blown and minimum standard core/miscellaneous clusters (subgroups "a", and "b", respectively) in groups FAS I and III. Values are means  $\pm$  SD.

| Primary variable                  | FAS Ia<br>(n = 26)       | FAS Ib<br>(n = 7) | FAS IIIa<br>(n = 19) | FAS IIIb<br>(n = 40) | P-value   |             |             |               |
|-----------------------------------|--------------------------|-------------------|----------------------|----------------------|-----------|-------------|-------------|---------------|
|                                   |                          |                   |                      |                      | Ia vs. Ib | Ia vs. IIIa | Ib vs. IIIb | IIIa vs. IIIb |
| Age (yrs)                         | 26.3 $\pm$ 4.6           | 22.4 $\pm$ 3.9    | 24.8 $\pm$ 4.7       | 27.7 $\pm$ 5.5       | NS        | NS          | 0.0194      | NS            |
| BMI (kg/m <sup>2</sup> )          | 21.5 $\pm$ 2.0           | 21.0 $\pm$ 1.9    | 31.4 $\pm$ 3.5       | 29.3 $\pm$ 6.0       | NS        | < 0.0001    | 0.0003      | NS            |
| Cutaneous androgenization [n (%)] | 19 (73.08)               | 4 (57.14)         | 15 (78.95)           | 28 (70)              | NS        | NS          | NS          | NS            |
| PFOs [n (%)]                      | 26 (100)                 | 7 (100)           | 19 (100)             | 40 (100)             | NS        | NS          | NS          | NS            |
| LH (mIU/mL)                       | 15.7 $\pm$ 3.7           | 12.4 $\pm$ 6.6    | 11.8 $\pm$ 3.1       | 12.3 $\pm$ 6.0       | NS        | 0.0004      | NS          | NS            |
| LH/FSH                            | 3.0 $\pm$ 0.9            | 2.2 $\pm$ 1.2     | 2.4 $\pm$ 0.5        | 2.6 $\pm$ 1.1        | NS        | 0.0236      | NS          | NS            |
| T (nmol/L)                        | 3.3 $\pm$ 0.7            | 2.6 $\pm$ 0.4     | 3.2 $\pm$ 0.8        | 3.0 $\pm$ 1.2        | 0.0132    | NS          | NS          | NS            |
| SHBG (nmol/L)                     | 35.3 $\pm$ 19.0          | 30.0 $\pm$ 23.4   | 13.0 $\pm$ 8.2       | 20.7 $\pm$ 18.3      | NS        | < 0.0001    | NS          | NS            |
| FAI (T/SHBGx100)                  | 12.0 $\pm$ 6.3           | 15.6 $\pm$ 11.7   | 36.5 $\pm$ 27.7      | 32.8 $\pm$ 36.9      | NS        | < 0.0001    | NS          | NS            |
| 17-OH-P (nmol/L)                  | 0* 5.7 $\pm$ 2.1         | 4.6 $\pm$ 1.1     | 5.4 $\pm$ 1.9        | 4.9 $\pm$ 2.4        | NS        | NS          | NS          | NS            |
|                                   | 1* 9.0 $\pm$ 3.4         | 8.3 $\pm$ 3.7     | 11.1 $\pm$ 3.3       | 11.0 $\pm$ 5.9       | NS        | NS          | NS          | NS            |
|                                   | $\Delta$ * 3.2 $\pm$ 2.5 | 3.7 $\pm$ 3.3     | 5.6 $\pm$ 2.4        | 6.2 $\pm$ 4.5        | NS        | 0.0013      | NS          | NS            |
| CYP21A2 [n (%)]                   | 8 (30.8)                 | 2 (28.6)          | 6 (31.6)             | 12 (30)              | NS        | NS          | NS          | NS            |
| DHEAS ( $\mu$ mol/L)              | 5.3 $\pm$ 2.1            | 7.6 $\pm$ 4.1     | 6.1 $\pm$ 2.0        | 7.1 $\pm$ 2.6        | NS        | NS          | NS          | NS            |
| Glucose (mmol/L)                  | 0* 4.5 $\pm$ 0.6         | 3.8 $\pm$ 0.6     | 4.7 $\pm$ 1.3        | 4.6 $\pm$ 0.8        | 0.0206    | NS          | 0.0174      | NS            |
|                                   | 1* 4.6 $\pm$ 1.2         | 5.1 $\pm$ 1.7     | 7.6 $\pm$ 3.1        | 7.3 $\pm$ 2.3        | NS        | 0.0001      | 0.0161      | NS            |
| Insulin (pmol/L)                  | 0* 43 $\pm$ 15           | 52 $\pm$ 27       | 129 $\pm$ 58         | 110 $\pm$ 167        | NS        | < 0.0001    | 0.0392      | 0.0122        |
|                                   | 1* 341 $\pm$ 129         | 245 $\pm$ 46      | 1139 $\pm$ 528       | 787 $\pm$ 473        | 0.0233    | < 0.0001    | 0.0001      | 0.0046        |

\* Determined in 8 out of the 14 volunteers; NS: not statistically significant. For abbreviations see Table 3.

other groups (FAS I to III), and C were not detected. Additional statistically significant differences of minor relevance among the groups are presented in Table 8, too. It has to be considered, however, that the number of patients were very small in this group, so that the statistical results have to be evaluated with caution.

#### Functional Androgenizing Syndrome (FAS) I (Ovary)

These patients were characterized by normally ranging BMI, elevated LH and LH/FSH values, hypertestosteronaemia and PFOs, respectively (Tab. 3, 8; Fig. 2). Serum LH levels were significantly higher in FAS I vs. all groups, and C. Highest LH and LH/FSH level were encountered in FAS Ia, and the two parameters were significantly elevated in FAS Ia vs. FAS IIIa (Tab. 9). Serum T levels were not significantly different among groups FAS I, II and III, but significantly higher in FAS I vs. FCA, FAS IV and C (Tab. 8; Fig. 2); furthermore, T levels were significantly higher in FAS Ia vs. Ib (Tab. 9). By definition, PFOs were visualized (at least unilaterally) in every FAS I patient. A linear correlation among T and DEAS levels in FAS Ib (miscellaneous ovarian-adrenal group) was not observed ( $P = 0.9$ ). There were no marked metabolic dysfunctions

found in that group, even if the value of Ins 1 was significantly higher in FAS I vs. C (Tab. 8; Fig. 2), and higher in FAS Ia vs. FAS Ib (Tab. 9). However, these values were within the means  $\pm$  SD of C, and markedly below the predefined cut-off level of Ins 1 (Tab. 2, 3). Patients in this group frequently suffered from cutaneous androgenization (ca. 70 %) (Tab. 8). Additional statistically significant differences of minor relevance among the groups are also presented in Table 8.

#### Functional Androgenizing Syndrome (FAS) II (Adrenal Gland)

This group is of complex nature. The following two major features were found (Tab. 3): in 3 out of the 10 FAS II patients, CYP21A2 mutations/deletions were associated with strongly elevated 17-OH-P levels whilst the residual 7 women were classified as FAS II patients mainly due to elevated DHEAS levels, T-levels being additionally elevated in 3 out of these 7 patients (Tab. 8). Although the means of 17-OH-P 0/1/ $\Delta$  showed highest serum concentrations in FAS II, the statistical power, however, was limited because of the enormous SD which can be explained by the tremendous increase of 17-OH-P levels in one of the FAS II patients; 17-OH-P 1 and  $\Delta$  were significantly higher in FAS II vs. FAS I,

and C. Highest levels of DHEAS were found in FAS II vs. all groups and C. Serum T were significantly higher in FAS II vs. FCA. As expected, both LH and LH/FSH were significantly diminished in FAS II vs. FAS I and FAS III, respectively. Two out of the ten FAS II patients belonged into subgroup FAS IIa where LH, glucose and insulin were obligatory in the normal range, the LH/FSH ratio was not increased and PFOs were not present; the disorder was caused by moderate and severe CYP21A2 mutations/deletions. Interestingly, in the first FAS IIa patient, the FSH/LH ratio was slightly increased and the ovaries were found to be small and oligofollicular (low antral follicle count) [55] indicating in this 28 yr old patient a STRAW -3 stage (late reproductive phase) [53]. The BMI in FAS II was significantly increased vs. FAS I and C and significantly lower vs. FAS III. Insulin 0 and 1 were higher in FAS II vs. C; however, the two variables were not significantly different between FAS II and III, a result which can be explained by elevated insulin levels in 4 FAS IIb patients (miscellaneous adrenal-obese-metabolic constellation) (Fig. 2); the same is true for the FAI level which was significantly higher in group FAS II vs. group FCA and C (Fig. 2); cutaneous androgenization was present in 80 % of the women. Addi-

tional statistically significant differences of minor relevance among the groups are also presented in Table 8.

#### Functional Androgenizing Syndrome (FAS) III (Multi-Organ-Disorders with FA, Obesity, Hyperinsulinaemia)

Patients of FAS III, the largest group, differ markedly from subjects of the other groups regarding several variables (Tab. 3, 8; Fig. 2, 3). The BMI of FAS III was significantly increased vs. FCA, FAS I, FAS II, and C. In correspondence, Gluc 1, Ins 0, and Ins 1 of FAS III were significantly elevated over vs. FCA, FAS I, and C, the difference reaching particular significance between FAS III vs. FAS I. Regarding Gluc 1, Ins 0, and Ins 1 largest differences were observed between FAS IIIa vs. FAS Ia (Tab. 9). Increased values of LH and LH/FSH ratio were found in FAS III vs. FCA, FAS II, FAS IV, and C. Groups FAS III, FAS I and FAS II share highest values of T, which were significantly increased in FAS III vs. FCA, FAS IV, and C. Levels of SHBG were lowest in FAS III vs. FCA, FAS I and C. In contrast, FAI values were highest in FAS III vs. FCA, FAS I and C. In addition, DHEAS levels were higher in FAS III vs. FCA, FAS I, and C, and 17-OHP  $\Delta$  were higher in FAS III vs. FCA, FAS I, and C. A linear correlation among T and DEAS levels in FAS IIIb (miscellaneous ovarian-adrenal-metabolic group) was not observed ( $p = 0.9$ ). By definition, PFOs were visualized (at least unilaterally) in every FAS III patient. In around 73 % of the subjects, cutaneous androgenization was observed (Tab. 8). Additional statistically significant differences of minor relevance among the groups are also presented in Table 8.

#### Functional Androgenizing Syndrome (FAS) IV (Residual Dysfunctions/Disorders with FA)

All 18 patients of this group (Fig. 1–3; Tab. 8) presented by definition at least one symptom of FA, e.g. cutaneous androgenetic symptoms and/or elevated levels of total T, FAI and/or DHEAS. In 11 patients, elevated BMI was associated with the presence of cutaneous androgenetic symptoms, and/or increased FAI and/or T levels, but without PFOs. The individual constellations found did not fit in any predefined clusters of either FCA, or FAS I to III. The high frequency of obesity in association with

lowered SHBG, increased FAI, and metabolic alterations, was the reason why significant differences between FAS III and FAS IV regarding BMI, SHBG, FAI, Gluc 1, Ins 0 and Ins 1 were not detected. In 3 additional cases, cutaneous androgenization and increased FAI in normal weight women were observed, again without the presence of PFOs. The combination of cutaneous androgenization and increased glucose or insulin levels without other pathologies were observed in 2 other patients. Another case showed an enhanced FAI in association with PFO. The presence of cutaneous androgenization, normal T and elevated DHEAS and insulin levels with PFOs was recorded in a further individual.

The distribution of the primary variables among the groups might be of additional interest.<sup>5</sup>

### Characterization

Detailed values of facultative variables (Tab. 2, 7) are shown for lipids only (Fig. 3); e.g. triglycerids were found to be significantly higher in FAS III vs. FCA, FAS I, FAS II and C and HDL levels were lower in FAS III vs. FCA, FAS I and C.

## Discussion

### General Remarks

The major message of this paper is that a paradigm shift in the understanding of female androgenization appears to be required. To achieve this aim i. a novel, markedly modified nomenclature was introduced; ii. an algorithm was setup using a new classification with clearly distinguishable pathological entities focusing primarily on the organs predominantly involved and comprising patients from puberty well into postmenopause; iii. a set of classifying variables were implemented distinguishing between primary (classifying) (e.g. elevated an-

drogen levels) [81] and secondary (“facultative”) variables; iv. corresponding to the new classification, four well-defined clusters were developed which were again differentiated into classic full-blown subsets (“a”) and non-classic minimum standard core subsets (“b”), thus establishing a stratification of reproducible plausibility; v. almost all definitions currently established in the field were integrated; and vi. final diagnosis is based both on grouping and patients’ individual phenotypes, making a flexible use of the classifying algorithm depending on patients’ current status, and altogether in consideration of the complex differential diagnosis.

The sequence of designation of the five groups was determined with clear intention. The special pathogenetic feature of the first three groups is characterized by the fact that the origin of the respective androgenetic dysfunction or disorder is caused predominantly by one organ, namely the skin (FCA), the ovary (FAS I), and the adrenal gland (FAS II), respectively. On the other hand, groups FAS III and IV belong together in some respects because obesity and metabolic alterations play an important role involving several organs in equal measure in these two groups. Even FAS IIb is very cognate to FAS III in a way. Although in both FAS I and III the ovarian dysfunction is concerning the pathogeneity of great importance, from the general therapeutic point of view, however, the internal affinity appears to be closer among FAS IIb, IIIa/b, and IV vs. FAS I and III.

The distribution of primary variables among the groups shows that most of them apply for more than one group. Here it should be pointed out that especially the occurrence of PFOs is common among most groups, howsoever pathologically distinct they may be. Thus, this particular variant of ovarian morphology, though hitherto considered a

<sup>5</sup> Cardinal features of FA were the presence of cutaneous androgenization, elevated levels of T, DHEAS, glucose and insulin as well as the visualization of PFOs (Tab. 3, 5, 6, 8, 9; Fig. 2). Elevated T levels were obligatory present in FAS Ia and b, and in FAS IIIa and optionally in FAS II and FAS IIIb, respectively. An PFO status was found constantly (by definition) in FAS Ia/b and FAS IIIa/b, and occasionally in FCA b, FAS IIb, or FAS IV. Elevated DHEAS may be present in FAS IIa/b, FAS Ib, FAS IIIb, and FAS IV, respectively; it was mainly found as an androgenic biochemical feature which appeared similarly to FA of ovarian origin, and was casually associated with obesity and/or metabolic dysfunctions, and in most cases with normally sized and normofollicular ovaries. Overweight and obesity were obligatory in FAS IIIa and optionally in FAS IIIb, FAS IIb or IV; an elevated BMI was not present in FAS I. Elevated glucose and/or insulin were obligatory found in FAS IIIa and optionally in FAS IIIb, FAS IIb and FAS IV; an elevation of these variables was missing in FAS I. *CYP21A2* mutation/deletion was an almost ubiquitous finding with strongest prevalence in FAS II (60 %) (detailed data are not shown).

key feature of female androgenization, is rather to be seen as a common, but not obligatory symptom [21]. For each group (apart from FAS IV), either a classic subset "a", or a non-classic subgroup "b" [61], casually appearing in a miscellaneous constellation, stand by. Subsets "a" are unique and match established comprehensible pathogenetic concepts, while subpopulations "b" are less uniform, but reflect more the wide range of multifaceted individual features. Subsuming FA patients under subgroups "b" limits the number of subsets that would otherwise increase immeasurably if such a bundling would not be made. Referring e.g. to the RCO3 [12, 13] at least 11 subsets are possible to be created [25, 30]. If taking all subsets of FA together one ends up inevitably with far more than 40 sub-phenotypes, an intention, that would be followed definitely with loss of clarity. Beside the just described novel issues in nomenclature and stratification of the entire FA, this is, to our knowledge, the first study comparing e.g. classic and non-classic, lean and obese FA ("PCOS/D")-patients. Furthermore, the intention has been turned to a not unimportant group of obese patients presenting biochemically non-adrenal androgenization without the presence of PFOs ("PCOs") (FAS IV). To our knowledge, this special group that might be equivalent to a patient group termed "Non-PCO-PCOS" [12, 13, 30] is never as exactly described as it is done in the present study.

An exactly repeatable diagnostic stratification is essential in order to guide customized treatment options by identifying patients' individual dysfunctions and disorders and by improving their risk assessment. Almost 20 years ago, Netter (1990) [21] recommended a similar imagination when he said: "A restructuring of the terminology might encourage a therapeutic approach that is consistently based on etiology." Admittedly, the herewith presented classification is not that easy to handle in daily clinical routine, it should be recognized, however, that it is the complex and widely ranged nature of female FA that determines the necessity of such a sophisticated logistic. It is the AESGO6 [30] that confirms this view. Of course, it is not mandatory that in daily routine the entire diagnostic procedure must be accomplished in each case. The herewith diagnostic approach should be embedded in a

modular worked out system. There is an entrance screening step I, and thereafter, in patients with either several and/or severe symptoms, particularly in young, obese and/or infertile patients, the second full diagnostic step II should follow. Either after step I or step II the specific therapeutic approach may result. Step III (genetic determinations; dexamethasone suppression test) should succeed, if necessary (full data are not shown and discussed here).<sup>6</sup>

Differences among the criteria of the NIH99 [134], the RCO3 [12, 13], as well as the AESGO6 [30, 31] on one hand, and the present systematics on the other hand are summarized in Table 10. The first three consensus criteria are alike in their structural composition, and they focus more and less all on the ovarian origin of FA. However, a clear definition both globally and in detail is missing so that the diagnosis "PCOS/D" get sometimes considerably out of hand into areas which can be definitely not referred on FA of ovarian origin [25, 29]. The present proposal brings the entire spectrum of FA together. Groups FAS I and III integrates principally the two major RCO3 subsets, and other, weaker defined subsets of the RCO3 are subdivided in other FA-groups.

#### Functional Cutaneous Androgenization (FCA) (Peripheral Androgenization)

This group covers all forms of cutaneous (peripheral) androgenization which are described as "idiopathic"[3, 5] meaning that other pathogenetic factors such as hyperandrogenaemia or disturbances of

the glucose/insulin metabolism are excluded. This diagnosis can be made independent on age, thus, in the cases e.g. of adolescent acne as well as of alopecia in older [72] postmenopausal women [68]. A noticeable number of local factors might be responsible on, such as 5 $\alpha$ -reductase [58], epidermic growth factor and others (see review: [7]).<sup>7</sup>

#### Functional Androgenizing Syndrome (FAS)

The following four groups which are of complex nature and therefore best characterized as a "syndrome" according to the AESGO6 [30], are ordained under the term FAS I to IV. Groups FAS I to III are arranged corresponding to the predominant organ (FAS I, and II) or a network of organs (FAS III) being involved. FAS IV is a not less important group of patients presenting androgenizing symptoms. This group let miss, however, a clearly dividing assignment or collects subsets which are not absorbed by the other groups.

#### Functional Androgenizing Syndrome (FAS) I (Ovary)

The pure classic subgroup, FAS Ia, is characterized by a cluster of symptomatology [8–10] which is clinically evident with full-blown expression during the early and peak reproductive phase (STRAW –5 to –4; [53, 135]; features are: normal BMI, missing marked metabolic deviations, elevated LH/FSH ratio [39, 74–79, 136], hyperandrogenaemia (hypertestosteronaemia) [5, 22, 39, 80] which is assumed to be an intrinsic abnormality in ovarian theca cell steroido-

<sup>6</sup> In order to ensure robust reliability regarding biochemical determinations a clear standardization is absolutely required [29]. Intracyclic, diurnal, perioral and episodic variations of circulating hormone concentrations should be methodically equalized as much as possible. In the present study, baseline blood sampling took place after an overnight (12 h) fast between 8 AM and 9 AM, and a pool value of three samples collected in 20 min intervals were used. Baseline blood samples were obtained in volunteers between day 3 and 5 of the menstrual cycle. In both patients and control enrolled in this study, estradiol and progesterone levels must reflect a menstrual status related to the early- to midfollicular phase excluding therefore any peri- or postovulatory phases. These definitions are of utmost importance regarding e.g. the evaluation of the LH/FSH ratio (see also below). The appropriate phase of the menstrual cycle should be considered prospectively in order to avoid that data of too many patients can not be evaluated accurately as it has been the case in our retrospective analysis. Furthermore, the current state of clinical assays are still not accurate and precise enough (e.g. concerning T measurement) [132]. It has been highlighted that some female samples give falsely high results of circulating T levels (> 3 nmol/L) determined in direct T immunoassays [133]; steroid compounds such as endogenous DHEAS or exogenous steroidal drugs might interfere with T-antibodies in the assay. In the present investigation, none of the participants has taken any drug, and in most patients with elevated DHEAS levels, the T values were < 3 nmol/L, which has been assumed to be the critical level.

There is no pretension that cut-off values used in the present study are universally applicable, however, they may represent a common international trend based on long-term experiences. Whatsoever, quantitative changes would not actually alter the principles of the algorithm proposed with the present investigation.

The same standardized timing as described for blood sampling is essential for sonographic visualization of the ovaries at which a functional menstrual state corresponding to the early-follicular phase might be the most reliable point in time as well.

<sup>7</sup> See next page.



**Table 10:** Comparison among NIH 1992 [28], Rotterdam consensus 2003 [12, 13], AESG 2006 [30], and the novel algorithm presented here.

| Term, tools                                                          | NIH                                                     | Rotterdam consensus                                     | AESG                                                    | Present systematics                                                  |
|----------------------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------------|----------------------------------------------------------------------|
| Head term                                                            | PCOS/D                                                  | PCOS/D                                                  | PCOS/D                                                  | Female androgenization (FA)                                          |
| Algorithm                                                            | –                                                       | –                                                       | –                                                       | +                                                                    |
| Areas                                                                | –                                                       | –                                                       | –                                                       | 3: A: functional A (FA)<br>○ B: tumorous A<br>○ C: pharmacological A |
| Primary target(s)                                                    | Ovary                                                   | Ovary                                                   | Ovary                                                   | General FA (skin, ovary, adrenal, multi-organ disorder)              |
| STRAW stages                                                         | –5 to –3                                                | –5 to –3                                                | –5 to –3                                                | –5 to +2                                                             |
| Groups/classes                                                       | –                                                       | 2 (without/with MetS)                                   | –                                                       | 5: FCA, FAS I–IV                                                     |
| Predefined subsets                                                   | –                                                       | –                                                       | –                                                       | Classic full-blown: a<br>Non-classic minimal core: b                 |
| No. of major clusters                                                | 1                                                       | 2                                                       | 1                                                       | 4                                                                    |
| Exactly defined variables                                            | Few                                                     | Few                                                     | Few                                                     | All                                                                  |
| Hierarchy of variables                                               | –                                                       | –                                                       | –                                                       | Primary and secondary variables                                      |
| Power of single variables                                            | Up to 50 %                                              | Up to 50 %                                              | Up to 50 %                                              | PVs: far less than 50 %<br>SVs: no classifying power                 |
| Oligo-, anovulation as variable                                      | Full parameter                                          | Full parameter                                          | Full parameter                                          | –                                                                    |
| Oligo-, amenorrhea as variable                                       | –                                                       | –                                                       | –                                                       | SV                                                                   |
| LH/FSH                                                               | –                                                       | –                                                       | –                                                       | PV                                                                   |
| OGLT                                                                 | –                                                       | –                                                       | –                                                       | PV                                                                   |
| Ovarian ultrasound feature                                           | –                                                       | PCO                                                     | PCO                                                     | PFO                                                                  |
| Possibility of poorly defined subsets                                | Several subsets exceed the primary definition of PCOS/D | Several subsets exceed the primary definition of PCOS/D | Several subsets exceed the primary definition of PCOS/D | Very limited                                                         |
| Group of residual cases                                              | –                                                       | –                                                       | –                                                       | Group FAS IV                                                         |
| Differentiation among classification, characterization and diagnosis | –                                                       | –                                                       | –                                                       | +                                                                    |
| Differential diagnosis                                               | Limited                                                 | Limited                                                 | Limited                                                 | Comprehensive                                                        |

NIH: National Institute of Health; AESG: Androgen Excess Society Guideline; STRAW: Staging Reproductive Aging Workshop [53]; MetS: metabolic syndrome; PV: primary variable; SV: secondary variable; OGLT: oral glucose loading test; for further abbreviations see Table 3.

genesis [137], as well as the visualization of PFOs (“PCOs”) [8, 55, 73, 125]. Cutaneous androgenetic symptoms, oligo-amenorrhea, and infertility are consecutive symptoms. Patients of group FAS I are equated with lean PCOS patients without the presence of the MetS as defined by the RCO3 [12, 13] and – independent on metabolic distur-

bances – defined by the AESGO6 criteria [30, 31], e.g. phenotype “A”: “Hyperandrogenemia”/“Polycystic ovaries”. Group FAS I (non-obese, lean “PCOS”) has been described before in several studies [39, 83, 98–100]. The etiology is unknown so far [23]. It seems that there is an intrinsic ovarian anomaly due to a born feature characterized by an elevated

density of preantral follicles in those ovaries [127]. A curative therapeutic concept does not exist [49]. However, one has to recognize that a genetically determined age-related depletion of the ovarian follicular apparatus [138–140] occurs with a reduction of the ovarian volume [141, 142]; the overall ovarian regression is possibly slower in “PCOS/

<sup>7</sup> Considering the RCO3 criteria [12, 13], FCAa patients who show an oligo- or anovulation (oligo- or amenorrhea) would be termed as “PCOS”-patients: “Clinical hyperandrogenism”/“Oligo- or anovulation”. Similarly, the AESGO6 criteria [30] would do so: e.g. group “F”: “Hirsutism”/“Oligoanovulation”. This example reveals again how poorly the term “PCOS/D” is defined [25], a fact which might be one of several reasons for misdiagnoses and misunderstandings which frequently occur still yet. Subgroups FCA a and b are mainly differentiated by ovarian morphology: patients with PFOs (“PCO”) [8, 55, 73, 125] are excluded in subgroup “a”, and accepted in subgroup “b” [125]. Adams et al. [125] have described similar patients who have revealed “multifollicular ovaries” but normal LH and T values. A slightly different subset of FCAb patients is observed sometimes presenting all features of FAS Ia (including an increased LH/FSH ratio) but without an elevation of androgens. The pattern of this special group of patients is comparable with the “asymptomatic PCO” [73, 134] or the “non-hyperandrogenic PCOS” [16, 31]. This subset is defined by the RCO3 criteria [12, 13] with: “Clinical hyperandrogenism”/“Polycystic ovaries”, or by the AESGO6 criteria [30] with: phenotype “E”: “Hirsutism”/“Oligoanovulation”/“Polycystic ovaries”, or phenotype “H”: “Hirsutism”/“Polycystic ovaries”. It has been stated in this context that the number of diagnosed PCOS increases using the RCO3 criteria [12, 13] because “women with ovarian dysfunction and polycystic ovaries, but without hyperandrogenism, were added to the PCOS diagnostic group” [15]. Taken together, FCA patients appear to compass an own group of subjects who should not be allocated under “PCOS/D”. This opinion is in agreement with the recommendation by Jonard and co-workers [73] who stated that an “asymptomatic PCO should not be considered as having PCOS”. Overall, the data suggest that the primary cause of cutaneous androgenetic symptoms and findings in group FCA is allocated on the level of the pilosebaceous unit. However, it is quite clear that this group covers itself numerous sub-phenotypes, e.g. papuloseous-pustuloseous acne vulgaris, frontoparietal androgenetic alopecia, combinations of two or three symptoms, dependency on age, and so on. Concerning the statistical relevance of this small group FCA it has to be considered that in this retrospective study patients of this groups were identified per exclusion; usually, a patient suffering e.g. from a simple acne vulgaris I° will not be examined exceeding the diagnostic screening step I.

D" patients [142] because of the larger pool of preantral follicles [126, 127]. This predetermined fate of ovarian aging coincides with gaining of regular menstrual cycles [135], and with a decline of testosterone levels in the middle of the 4<sup>th</sup> decade of life [143] of "PCOS/D" patients. Hence, a partly self-healing process of the "PCOS/D" (especially: FAS I and III) can be assumed. Here, it should be recognized again (compare above) that the PFO ("PCO") status presents just a symptomatic expression of a spectrum of ovarian morphology being associated with several functional conditions: the so called "polycystic ovary" is a "marker that is itself unspecific" [24].

In addition, it has been elaborated in the present study that there are, apart of "classic" FAS Ia patients, some women with predominant ovarian origin of C<sup>19</sup>-sexual steroid excess who possess merely the minimum standard core cluster (FAS Ib) ("non-classic PCOS") [61]; this contains three obligate variables, namely: i. hypertestosteronaemia, ii. normal BMI, and iii. PFOs ("PCOs"). Group FAS Ib considers, for instance, the perception of the RCO3 [12, 13] that an elevated LH/FSH ratio is not "required or useful" for making the diagnosis "PCOS/D". In contrast to the RCO3 [12, 13] and the AESG06 [30], however, we strongly promote, that circulating LH and LH/FSH ratio should serve as primary variables. Nevertheless, groups FAS Ia and b encompass the two subphenotypes, namely ovarian FA with and without increased LH/FSH ratio.<sup>8</sup>

The introduction of FAS I as an own entity in the present classifying strategy

coincides with Baillargeon's statement [149] that "some patients with PCOS are neither insulin resistant nor hyperinsulinaemic", or with DeUrgate et al. [150] emphasizing that "although insulin resistance is a common abnormality in PCOS, it does not seem to be a universal feature". It has to be conceded, however, that FAS I is associated with a slight, but significant increase of Ins 1 (post oGTT) vs. C, a result which veers forward findings observed by others as well [109, 151]. The significant increase of insulin within the SD of the control points out that these patients are under a very low risk to develop a relevantly pathogenetic level of hyperinsulinaemia that might result in impaired glucose tolerance and type 2 diabetes [97] over time. Actually, there are no longitudinal studies demonstrating that this highly selected group of patients may follow such a perspective either after weight gain or even in keeping their normal body weight as also stated by the RCO3 [12, 13] (Tab. 2). In addition, the association of FAS I patients with risk of gestational diabetes [152] is very unlikely.

The background for introducing FAS Ib is intensively described in Material and Methods (Novel nomenclature and algorithm of classification, characterization and diagnosis, FAS I).

Summarizing the data of group FAS I, it is very presumable that the primary origin of the entire spectrum of dysfunctions of this group is predominantly the ovary; cutaneous androgenetic symptoms, disturbed pituitary function, cycle irregularities, as well as infertility appearing to be secondary,

and metabolic disturbances might be negligible.

#### Functional Androgenizing Syndrome (FAS) II (Adrenal Gland)

Unfortunately, both the RCO3 and the AEGO6 fail to suggest a detailed algorithm for the differential diagnosis between ovarian and adrenal androgen oversecretion; by the way, the apparent lack of developing an assortative structure for the FA II group is related to the entire literature. This group consists of two major different constellations: i. a phenotype caused by *CYP21A2* mutation/deletions (additional genetic mutations/deletions are not investigated here) which result in biochemic and clinical FA appearing for the first time in the peri- and postpubertal phase, in adolescence or in adult life [35, 36]; ii. elevated DHEAS levels without traceable genetic defects [95] apparently due to mild 3 $\beta$ -hydroxysteroid dehydrogenase deficiency [91] or an exaggerated adrenocortical  $\Delta^5$ -17-hydroxylase activity [92]. In addition to these conditions, the pure group FAS IIa is characterized by normal (or even decreased) LH/FSH ratio, normal glucose and insulin levels as well as by a non-PFO ("non-PCO") status. These findings prove the existence of a "pure" adrenal FA independently of any primary ovarian dysfunction or aging.<sup>9</sup> Particularly, the FAS IIa patient with elevated FSH/LH ratio as well as reduced ovarian size and low antral follicle count [(s. Results, Functional Androgenizing Syndrome (FAS) II (Adrenal Gland)] support this concept. Thus, the current hypothesis that – in any case – adrenal hyperandrogenaemia is a prerequisite of ovarian androgen overproduc-

<sup>8</sup> Barth et al. [29] do have the opinion that it "is a good decision" that "the Rotterdam consensus has discarded the use of LH and FSH ratios as diagnostic criteria" because the positive predictive power of LH measurement in the whole population appears to "fall to 3–6 %". But this conclusion is anyhow somewhat equivocal because it has been stated by the same group [8] that "elevated LH concentrations can be observed in 40–60 % of PCOS women" and that: "... an elevation in serum LH concentrations is pathognomonic of PCOS, ...". In the study by Escobar-Morreale et al. [144] using receiver operating characteristics, it was found that LH failed to reveal differences among PCOS patients and non-affected individuals. However, the means  $\pm$  SD of LH and FSH in PCOS patients vs. non-affected subjects have been higher in the latter group ( $8.1 \pm 5.2$  and  $7.4 \pm 2.9$  vs.  $10.2 \pm 11.0$  and  $11.8 \pm 18.2$ , respectively). This result suggests that there has been some bias both in timing of blood sampling and in selection of control individuals in this study. Indeed, the finding of elevated LH concentration and increased LH/FSH ratio is present solely in subsets of WHO II ovarian insufficiencies (out of the entire range of WHO I to VII ovarian insufficiencies) [27] which correspond exclusively to groups FCAb, FAS I, FAS IIb and FAS III. All these groups are associated with PFOs ("PCO") and/or FA; in consequence, the multiple regression showed statistically significant correlation of PFO and LH levels in the present study. This fact goes along with strong evidences indicating that hypersecretion of LH is secondary to ovarian factors in "PCOS/D" patients [76, 77, 145]. Marshall et al. [146] have stated recently that "evidence to date has failed to identify a primary hypothalamic abnormality and suggests that the observed abnormalities of GnRH secretion are secondary to the abnormal hormonal milieu in plasma", and further that "existing data suggest that hyperandrogenemia may play a central role in the evolution of altered hypothalamic sensitivity to ovarian steroid feedback during normal pubertal maturation". It appears that the weakness of definition of "PCOS/D" [25, 29] and the lack of standardized blood sampling [29] (compare above) rather than the symptom of hypersecreted LH itself is responsible on a certain loss of its predictive value. Our findings that both LH and LH/FSH levels tend to result in highest concentrations in group FAS I comparing to the other groups and found to be significantly higher in FAS I vs. FAS III, and, particularly in FAS Ia vs. FAS IIIa patients, agree with the observation made before by our group [39] and by Antilla et al. [147]. These authors have demonstrated that lean normoinsulinaemic "PCOS/D" patients have higher LH levels than lean or obese hyperinsulinaemic PCOS/D patients. Baseline LH levels, LH pool and LH pulse amplitude are inversely correlated with BMI [78, 148]. The increase of circulating LH levels is highly associated with the rate of infertility and cycle disturbances [86].

<sup>9</sup> See next page.

tion coincident with the presence of PFOs ("PCO") [75, 80] appears to be disproved with this. Corresponding to this idea, Azziz and colleagues reported previously as well [43] that excess circulating levels of the adrenal C<sup>19</sup>-sex steroid, DHEAS, in group FAS II reflect an intrinsic abnormality of adrenocortical steroidogenesis independent of ovarian function. An age-related decline of DHEA-S levels [71, 89, 93, 94] has to be taken into account indicating a partly self healing process with ageing (compare: Discussion/Functional Androgenizing Syndrome [FAS] I [Ovary]). Furthermore, extra-adrenal factors such as DHEA sulfotransferase might play an additional role in increased levels of DHEAS [95]. However, the suggestion, that an increase in DHEA sulfotransferase activity might be secondary to hyperandrogenaemia did not apply in 4 out of the 7 FAS II patients whose elevated DHEAS levels were not associated with elevated T levels indicating that the enhancement of DHEAS levels was actually adrenal rather than extra-adrenal origin in these patients. In congruence, a correlation among DHEAS and T levels was not found either in generally nor in groups FAS Ib and FAS IIb (miscellaneous constellation). By the way, the dexamethasone suppression test [2, 153] appears to be still useful for the exclusion of an androgen producing tumour (differential diagnosis between areas A and B) (step III of diagnosis) (Fig. 1).

Group FAS IIb includes patients with miscellaneous constellations, e.g. adrenocortical-ovarian dysfunctions [42, 95, 154–156] or adrenocortical FA with overweight and/or metabolic disturbances (Tab. 3) [101, 156]; the adrenal part is supposed to be the dominant one in this group, particularly regarding individual therapeutic procedures. Therefore, the FAS IIb subset is responsible on enhanced values of FAI and Insulin 1 levels in group FAS II (Tab. 8; Fig. 2).

#### Functional Androgenizing Syndrome (FAS) III (Multi-Organ-Disorders with FA, Obesity, Hyperinsulinaemia)

Several studies have reported on this group of patients before [39, 41, 83, 98–101]. Full-blown characteristics indicate that a dysfunctional network of multiple organs is involved (e.g. ovaries, pituitary, skin, fatty tissue, pancreas, liver) resulting in symptoms and dysfunctions such as PFOs, hypertestosteronaemia, increased LH/FSH ratio, oligomenorrhea, obesity, hyperinsulinaemia, and infertility. Almost pathognomonic for this group of patients is the significant reduction of SHBG levels [98, 107, 110–115]. Subjects of group FAS III appear to be equivalent with those RCO3-PCOS patients who show symptoms of the MetS [12, 13]. In fact, impressive are the data of FAS III concerning the highly significant prevalence of elevated 1-h post-load glucose as well as fasting and 1-h post-load insulin levels vs. the other groups; in this regard, the comparison between FAS IIIa and FAS Ia is of especial significance. Using multiple regression analysis, both BMI and FAI showed a statistically significant correlation with fasting and 1-h post-load levels of insulin. Merely BMI indicated a significant correlation for fasting and 1-h post-load levels of glucose. These models explain 15 % and 31 % of the variability of glucose 0 and 1. Addressing the issue of determination of glucose and insulin levels using the OGLT, the present study supports former investigations [39, 106] that the risk of prediabetes is determined properly by using the simple 60-min oGLT including the determination of both glucose and insulin; in obese females, the prevalence of values exceeding the 3<sup>rd</sup> quartile of the normoweight control was found to be 91.5 % using the 60 min OGLT vs. 73.4 % using the HOMA-IR [106]. If one counts the data of glucose and insulin additionally to the results of lipid analysis of FAS III (Tab. 8, 9; Fig. 2, 3) the differences become even stronger in comparison with the other groups. Maternal obesity

was found to be an independent risk factor for spontaneous abortion after oocyte donation [157], and a meta-analysis has elaborated that maternal obesity is associated with an increased risk of congenital anomalies [158]; and hyperinsulinaemic "PCOS" patients appear to be at increased risk of developing gestational diabetes [152]. Because it is known that the incidence of type 2 diabetes is predicted by fasting glucose and insulin [159], the conversion from normoglucaemic to impaired glucose tolerance and type 2 diabetes in "PCOS" patients [160] should be paid considerable attention.

Obesity and insulin resistance are highly associated with MetS, a cluster that itself has a high relative risk of type 2 diabetes and cardiovascular disease as found in a 7 to 11 yr follow up study [161]. In the present algorithm, symptoms of MetS were integrated by a combination of primary and secondary variables; unfortunately, the frequency of MetS in our patients can not be specified completely, since blood pressure measurement, one major parameter of MetS [12, 13, 118, 119], was not performed regularly in all of our patients, particularly not in the younger. However, the results of lipid determinations themselves (Fig. 3) suggest a marked accumulation of risk for cardiovascular disease in FAS III (and in FAS IIb and FAS IV) patients as similarly found before [39]. In individuals with severe obesity and hyperinsulinaemia, an acanthosis nigricans [162] is frequently found, a pathologic constellation which might be equivalent with the term "hyperandrogenaemia – insulin resistance – acanthosis nigricans (HAIR-AN)" syndrome [52, 163]. Obese "PCO" patients with AN were found to reveal highest insulin levels [164]. Acanthosis nigricans appears to be a consecutive symptom due to hyperinsulinaemia and it ranks therefore as secondary (facultative) variable in the present diagnosis system (data not shown).

<sup>9</sup> Asymptomatic carriers of *CYP21A2* deficiencies are found in approximately 85 % in heterozygosity [90]. If there is no phenotypic adrenal FA (FAS II) present, or in other words, if an adverse geno-phenotype translation has not taken place, then these genetic defects do not have any impact on grouping because of the unspecifically wide spread among the entire population [18] as it has been confirmed in the present study (detailed data not shown). If clinical symptoms appear firstly at puberty or later in life then the term "FAS II" rather than "NC-CAH" or "late onset AGS" should be used (compare: Introduction, particularly FN1). Group FAS II is another example showing that is absolutely required to go for a compromise among the putative number of grouping set-ups and patients' individualization: a strict splitting into numerous subsets would make the entire regimen too complicate, and the number of patients per group would be too small so that a statistic comparison would be not possible anymore among the groups in most cases. From the daily clinical point of view, it is, on the other hand, a clear disadvantage anyway to focus exclusively on either ovarian or adrenal androgenization, thereby the overview is lost, particularly concerning the right therapeutic strategies which should follow.

Minimum standard sub-phenotypes were assigned to group FAS IIIb: at least one androgenizing symptom, either an increased BMI or alterations of circulating glucose and/or insulin levels were found, and all patients have got PFOs; LH, LH/FSH and SHBG levels were variable. These subsets might have overlapping features with phenotypes described as “metabolically healthy but obese (MHO)” patients [165] or as “metabolically obese but normal-weight (MONW)” individuals [166, 167], respectively. Interestingly, MHO subjects are at less risk of type 2 diabetes and cardiovascular disease than MONW subjects [161]. These data indicate again that the determination of insulin is of importance concerning an individual risk evaluation [25]. In correspondence to the miscellaneous ovarian-adrenal FAS Ib phenotype, FAS III pattern patients with elevated DHEAS levels were grouped into FAS IIIb. An increased activity of the hypothalamic-pituitary-adrenal axis in obese “PCOS” patients has been described [168]. DHEAS levels were found to be significantly higher in the (obese) group FAS III vs. the (lean) group FAS I (Tab. 8), however, looking at the subgroups “a” and “b” these differences were not present anymore (Tab. 9). A DHEAS accentuation as an example of the predetermined variability in FA was observed in FAS IIa, IIb, Ib, FAS IIIb and FAS IV, respectively.

Summarizing the data of FAS III, it becomes very clear, that the largest group in this study represents an own complex spectrum among the FA-entities. The importance of detecting irregularities of the glucose and insulin metabolism as early as possible is incremental alone because of the fact, that there is an increasing prevalence of overweight among children [169]. In this respect, the evaluation of the present data does not agree with the consequence of the AESO6 statement [30] declaring that the feature “insulin resistance” has “not formed part of any of the recognized definitions to date.”

#### Functional Androgenizing Syndrome (FAS) IV (Residual Dysfunctions/Disorders with FA)

This group integrates some further naturally occurring sub-phenotypes of FA, which, to our knowledge, have been never before described as exactly as it is

done in the present study. Frequent exponents of FAS IV are obese women with cutaneous androgenization, normo-androgenaemia, enhanced FAI and normal or small sized ovaries with normo- or oligofollicular state; the androgenization might be explained through the reduction of SHBG induced by obesity or hyperinsulinaemia [98, 107, 110–116] and resulting in an increased FAI (compare Tab. 6). Even functionally older women (STRAW  $\geq -2$ ) [53] might belong to FAS IV. This group might have got overlapping features with the “non-PCO-PCOS”, an absolutely illogical term; criteria of the NIH, the RCO3 and the AEGO6 [30] declare those constellations as PCOS phenotypes “B”, “D”, and “F”: “Hyperandrogenaemia”/“Hirsutism”/“Oligoanovulation”, “Hyperandrogenaemia”/“Oligoanovulation”, and “Hirsutism”/“Oligoanovulation”, respectively. As already discussed several times above, the weak and misleading definitions of “PCOS/D” are not really helpful for our understanding and might “continue to plague the PCOS scientific literature” [30]. Other androgenized patients of group FAS IV let miss a clearly deviding assignment; grouping is performed by exclusion. For example, the combination of cutaneous androgenetic symptoms and increased glucose or insulin levels without other pathologies match this group. It should be emphasized that the assignment of findings in group IV happened randomly in contrast to FCA and FAS I–III where a predefined allocation took place as described above. Group FAS IV was an exclusion group related functionally among group FCA and groups FAS I–III on one hand, and “differential diagnosis” (s. below) on the other hand.

#### Differential Diagnosis

Both the diagnosis of “FCA” and “FAS” and their differential diagnosis (Tab. 4) provide a profound knowledge on all the varieties of dysfunctional states and morbidities which might be associated with symptoms of FA. The differential diagnosis has to exclude e.g. dermatologic, internistic or monogenetic disorders being associated with androgenetic or associated symptoms which do play a minor role in the context of the underlying disease, or do not have got any endocrine etiologies or androgenetic relationships (compare: Material and Methods, Differential Diagnosis).

#### Dynamic Shifts among the FA Groups and in Dependency on Patients’ Current Status and Ageing

The grouping of patients described here is not static in the sense that a group assignment, once carried out, will be valid for ever. The boundaries are fluent, and therefore it may be possible that a passing over among the groups may occur in dependency on exogenous circumstances or altered conditions during female life from puberty to menopause.<sup>10</sup>

Ageing will be accompanied diversely with group transitions. For instance, a FAS Ia patient will change over to a FAS Ib patient with the conversion from an elevated LH/FSH ratio to an increased FSH/LH ratio during menopausal transition (STRAW stages  $-2$  to  $-1$ ; [53]. In parallel with age-dependent follicular depletion and consecutive normalisation of pathologic factors discussed above (see: Discussion, FAS I), a further group shift into FCAb will take place; and will pause in this group later on, if e.g. the hirsutism is irreversibly manifested. Or, hirsutism might be a life-long symptom, clinically evident in adolescence as a variable of FAS IIIa which might remain the only irreversible symptom of FA up to postmenopause, and might be then classified as FAS IV together with the final diagnosis of MetS.

In contrast to FAS I, a FAS II constellation, in the case of a translation-effective *CYP21A2* mutation/deletion, may continue lifelong even when the physiologic change from STRAW  $-3$  to  $+2$  has occurred [53]. On the other hand, it

<sup>10</sup> Lean patients of group FCAb are functionally very closely related to those of FAS Ib, and grouping is dependent on whether elevated T-levels are observed or not later on. With weight gain which is caused possibly through a psychosomatic crisis, FAS I passes over to FAS IIIb, and when obesity is consequently followed by hyperinsulinaemia (insulin resistance) [101], FAS IIIb will end up as FAS IIIa. The other way around is also conceivable: weight loss is accompanied with normalization of metabolic dysfunctions through life style modulations [170], and such a course may lead to a conversion from FAS IIIa to FAS Ia. While this transition will take place relatively easy, a complete disappearance of FA, however, will happen very delayed or more likely not at all, because the PFO status, an obligate primary (classifying) variable of the two groups, FAS I and III, is irreversible during reproductive life (STRAW stages  $-5$  to  $-3$ ; [53] as a rule. The possibility of conversion among “classical PCO” and “idiopathic hirsutism and vice versa” has been already discussed previously by Rittmaster et al. [1].



could be also possible, that FAS II in the case of elevated DHEAS levels disappears with menopausal transition due to the age dependent decline of DHEAS [93, 94], or is translated to FCA when e.g. the hirsutism remains.

## Conclusion

The study presented reveals for the first time that a reproducible algorithm with a well balanced compromise of classification and individual characterization over the entire range of FA from female puberty to postmenopause including dermatologic, gynaecologic, internistic and molecular genetic issues is applicable. It should be emphasized that the exactly defined classic “a” constellations may serve as ideal models for further research (e.g. for prospectively randomized controlled studies) on different FA entities. Further studies of our group are on the way to elucidate more details (e.g. impact of anti-muellerian-hormone on grouping) of the different well defined features particularly regarding various therapeutic target points. Currently, the development of an electronic version of a medical report is on the way, that will generate half-automatically and flexibly the final diagnosis based on both the entered data cluster and the individual constellation. Such an electronic tool will support the clinical usefulness and reproducibility of the presented systematic regarding the appropriate evaluation and treatment of female functional androgenization.

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